Selective Serotonin Reuptake Inhibitors Aggravate Depression-Associated Dry Eye Via Activating the NF-κB Pathway

Xiaohao Zhang,1–3 Yue Yin,1–3 Ling Yue,4 and Lan Gong1–3

1Department of Ophthalmology and Vision Science, Eye, Ear, Nose, and Throat Hospital of Fudan University, Shanghai, China
2NHC Key Laboratory of Myopia (Fudan University), Laboratory of Myopia, Chinese Academy of Medical Sciences, Shanghai, China
3Shanghai Key Laboratory of Visual Impairment and Restoration, Fudan University, Shanghai, China
4Shanghai Mental Health Center, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Correspondence: Lan Gong, Eye, Ear, Nose, and Throat Hospital of Fudan University, Room 405 Building 10, No. 83 Fenyang Road, Shanghai 200031, China; 13501798683@139.com.
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PURPOSE. Our study aimed to evaluate the side effects of selective serotonin reuptake inhibitors (SSRIs) on the ocular surface.

METHODS. Twenty patients with depression and dry eye disease (DED) were randomly picked to receive SSRI treatment, whereas another 20 patients received placebo treatment. The serotonin, inflammatory cytokine, and proapoptotic protein levels were determined by using protein chip, qRT-PCR, and ELISA analyses. A rat depression model was established, and SSRIs were applied for 3 or 6 weeks. Tear production and corneal epithelial barrier function were evaluated. The serotonin and inflammatory cytokine levels were analyzed by qRT-PCR, immunohistochemical staining, and ELISA. Human corneal epithelial cells were subjected to serotonin, a HTR antagonist, and/or an NF-κB signaling inhibitor. The inflammatory cytokine and proapoptotic protein levels were determined by qRT-PCR, Western blot analysis, and ELISA. The cell apoptosis rate was assessed by using flow cytometry.

RESULTS. The SSRI group had higher tear serotonin levels and more serious inflammation and cell apoptosis on the ocular surface. In the rat depression model, depression decreased tear secretion and increased IL-1β and TNF-α production, whereas the serotonin, TLR2, and TLR4 levels were not increased. SSRI-affected the corneal epithelial barrier, and promoted an inflammatory response on the ocular surface by increasing the tear serotonin levels. In addition, serotonin induced an inflammatory response and cell apoptosis in corneal epithelial cells by activating NF-κB signaling.

CONCLUSIONS. SSRIs aggravate depression-associated DED via activating the NF-κB pathway. The antagonist of HTRs or the inhibitor of NF-κB signaling presents a potential therapeutic strategy for depression-associated DED. (Trial registration number, ChiCTR1800015592).

Keywords: SSRIs, depression, dry eye disease, serotonin, NF-κB pathway

Dry eye disease (DED) is a common multifactorial inflammatory condition that leads to visual disturbance, chronic ocular discomfort, and a significantly poor quality of life.1 DED affects 10% to 30% of the population and has staggering healthcare costs, verifying that it is a major public health concern.2 Although the pathogenesis of DED is not fully understood, tear deficiency and ocular surface inflammation are well established as major contributors. Owing to its profound and widespread influences, fully realizing the pathogenesis of DED is necessary.

Clinical epidemiologic studies3–7 have found that DED frequently occurs in patients who have depression and/or anxiety disorders, especially patients who are older. One of the most commonly prescribed medications for the treatment of depression is selective serotonin reuptake inhibitors (SSRIs), which inhibit serotonin (5-HT) transport. Accumulating evidence suggests that SSRIs are closely related to the risk of DED.8,9 Benzodiazepines and some other antidepressants, including tricyclic, noradrenergic, and specific serotonergic antidepressants, are not associated with DED.1 Importantly, our previous study10 indicates that SSRIs are independent risk factors for DED. Patients using an SSRI have a 1.551-fold increased risk of DED compared with that of patients using other types of psychotropic medication or not using these medications.10 Some studies11–13 have also reported that patients with DED have higher tear serotonin levels. Therefore, DED caused by SSRIs should receive more attention.

SSRIs inhibit the transport of 5-HT, which can increase extracellular serotonin concentrations.12 The efficacy of SSRIs most likely depends upon the brain’s adaptive response to sustained increases in the 5-HT level. Abnormally increased extracellular 5-HT levels may induce some serious side effects. Accumulating evidence has confirmed that prolonged SSRIs can activate the peripheral TLR4 signal pathway and NF-κB signaling.13,14 TLR4 is a transmembrane protein receptor that is involved in innate immunity. TLR4 signaling can activate many inflammatory cytokines that have been proven to be triggers of DED.15 5-HT binding to 5-HTRs can activate NF-κB.13 As a transcriptional activator, NF-κB increases the expression levels of downstream genes, including the inflammatory cytokine genes TNFα and TLR4 and the apoptosis-related genes AIF and
BAX, and provokes a rapid inflammatory response and cell apoptosis. However, the mechanism by which SSRIs induce DED is not clear. Further studies are necessary to explore the effects and mechanism of SSRIs in DED.

In this study, we showed that SSRIs induce ocular surface damage and aggravate depression-associated DED via activating the NF-kB pathway. Importantly, DED caused by SSRIs can be rescued by an antagonist of HTRs or inhibitor of NF-kB signaling. Our findings provide new insights into the pathogenesis of DED and present a potential therapeutic strategy for depression-associated DED.

METHODS

Subjects
This study was conducted in compliance with the Declaration of Helsinki for research involving human participants. The study was performed at the Shanghai Eye and ENT (EENT) Hospital and psychiatric clinic of the Shanghai Mental Health Center, China. All consecutive patients with dry eyes and depressive disorders (age ≥16 years) who were treated between May 1, 2017, and May 1, 2018, were enrolled in the study. The study design was approved by the Ethics Committee of Shanghai EENT Hospital, and all subjects gave written informed consent. Forty patients were randomly divided into two groups. Twenty participants underwent SSRI treatment, and the other patients underwent psychological treatment. At the same time, a group of matched people without depression and DED that did not receive any treatment served as the untreated normal group. The participants were assessed on the day before treatment and 90 days after treatment. The patients were questioned about their demographics, history of systemic diseases, and medication. All patients completed the self-rating anxiety scale (SAS), self-rating depression scale (SDS), and ocular surface disease index (OSDI) questionnaires during the interview and before the ophthalmic examination. The ophthalmic symptoms were queried, and a slit-lamp examination was performed. Then, Schirmer’s I test (SIT), tear break up time (BUT) test, corneal fluorescence staining (CFS), meibum quality, and meibomian gland expressibility were evaluated.

Tear Fluid Washings
Tear fluid (0.5 μL) was collected with a 0.5-μL glass capillary tube (Drummond Scientific, Broomall, PA, USA) by capillary action from the inferior tear meniscus of each eye. Tear samples from both eyes (1 μL total) were eluted into a sterile tube containing 9 μL PBS and 0.1% bovine serum albumin (Sigma-Aldrich Corp., St. Louis, MO, USA). The tubes were stored at −80°C before conducting the activity assays.

RNA Isolation
Gene expression in the conjunctival epithelium was evaluated in 20 SSRI, 20 control, and 20 normal subjects. Conjunctival epithelial RNA enriched for mRNA was isolated from impression cytology samples by using lysis buffer, followed by selective binding of RNA to the silica gel–based membrane (RNeasy Micro Kit; Qiagen, Gaithersburg, MD, USA). The RNA concentration was measured by its absorption at 260 nm, and the samples were stored at −80°C before performing polymerase chain reaction (PCR).

Protein Chip
The L-based Human Antibody Array 507 (H-Wayen Biotechnologies, Shanghai, China) was used to screen for differential expression of more than 500 proteins, including cytokines, chemokines, inflammatory mediators, growth factors, and matrix metalloproteinases. In this study, 20 patients with depression and DED were randomly picked to receive SSRI treatment, whereas another 20 patients received placebo treatment. After 3 months of SSRI treatment, we collected the tear samples of everyone in the two groups. We randomly selected four samples from each group to analyze the kind and content of proteins in tears by protein chip, following tear collection for 24 hours. Supernatants were collected by centrifugation at 2000g for 10 minutes and were tested by the protein array company.

Animals
Male Sprague-Dawley rats weighing 150 to 200 g upon arrival were individually housed under constant temperature (23°C ± 2°C) and a 12 hour/12 hour light/dark cycle with free access to food and water before initiation of the experiment. Eighty rats were randomly divided into four groups as follows: one group of 20 rats underwent chronic unpredictable mild stress (CUMS), one group of 20 rats underwent CUMS and SSRI treatment, one group of 20 rats underwent SSRI treatment, and one group of matched rats did not receive any treatment (untreated control rats). All animal procedures were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research, and the experimental protocol was approved by the Experimental Animal Ethical Committee of EENT of Fudan University.

Depression Model Establishment
The CUMS protocol was adapted from a previous study. Briefly, the rats were subjected to different mild stressors for 28 days. The control rats were handled daily without any stress in the housing room. On postoperative day 28, the percentage of 1% sucrose consumption within 24 hours was calculated as follows: percentage (%) = total sucrose consumption/total water consumption × 100. The forced swim test was performed as previously described on postoperative days 25 to 26 between the hours of 12 noon and 3 PM. The time spent swimming, straining, and immobile and the latency to immobility were analyzed by using a swimming analysis system. On postoperative days 22 to 24 between the hours of 1 PM and 4 PM, spontaneous behavior and exploration were analyzed by using the open-field test as previously described. Briefly, spontaneous behavior was recorded by using a detection system (MED-VOF-RS, Institute of Brain Science, Fudan University, Shanghai, China) in a quiet room. The total distance, central duration, and frequency explored during the 5-minute test were analyzed.

SSRI Paroxetine Treatment and Index Measurement
After CUMS, the rats (n = 20 per drug treatment group and n = 20 per CUMS and drug treatment group) received the SSRI paroxetine (Sigma-Aldrich; 10 mg/kg/d) for a 6-week period via their drinking water according to a previously published protocol. The index measurement was performed at 0, 3, and 6 weeks of SSRI paroxetine treatment. The comprehensive ophthalmic examination included a slit-lamp examination, Schirmer’s test, and corneal lissamine green staining. The aqueous tear volume was measured with the phenol red thread (Zone-Quick; Yokota, Tokyo, Japan) tear secretion test at 1 PM in the standard environment. Tear fluid (0.5 μL) atraumatically collected from the inferior tear meniscus of each eye (with
tears from four eyes representing one group) was stored at −80°C. Total RNA collected from the cornea was extracted by using a purification kit (RNeasy Micro Kit; Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. The eyes of the rats were excised and embedded in paraffin compound at 0, 3, and 6 weeks of SSRI paroxetine treatment, and the paraffin-embedded samples were cut into sagittal sections (8-μm thick).

**Immunohistochemistry**

Immunohistochemistry was performed by using the peroxidase-conjugated avidin-biotin method. Briefly, tissue sections were incubated with antibodies specific for TLR4 (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA), IL-1β (1:1000; Abcam, Cambridge, MA, USA), TNF-α (1:200; Abcam) at 4°C overnight, followed by incubating with appropriate secondary antibodies. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI), and photomicrographs were taken under a laser scanning confocal microscope (Nikon, Tokyo, Japan).

**Cell Culture**

Human corneal epithelial cells obtained from the RIKEN BioResource Research Center (Tokyo, Japan) were cultured in Dulbecco’s modified Eagle’s medium/nutrient mixture F-12 (DMEM-F12; HyClone, Logan, UT, USA) supplemented with 8% fetal bovine serum (Gibco, Grand Island, NY, USA) and maintained at 37°C in a 5% CO₂ and air-humidified incubator.

**Western Blot Analysis**

The cells were extracted with cold RIPA buffer (Beyotime, Beijing, China). The cell lysates were standardized on the basis of protein content. Equal protein lysates (30 μg/lane) were separated by 10% SDS-PAGE and blotted onto polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). The membranes were incubated overnight at 4°C with primary antibodies, including IL-1β (1:1000; Abcam), IL-6 (1:1000; Proteintech, Wuhan, China), IL-10 (1:500; Abcam), TNF-α (1:500; Abcam), TLR2 (1:1000; Abcam), TLR4 (1:200; Santa Cruz Biotechnology), AIF (1:1000; Proteintech), BAD (1:1000; Santa Cruz Biotechnology), AIF (1:1000; Proteintech), BAX (1:1000; Proteintech), P-p65 (1:2000; Abcam), p65 (1:2000; Abcam), IκBα (1:1000; Abcam), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:12000; Proteintech). The next day, the membranes were incubated with peroxidase-conjugated secondary antibodies (1:5000; Proteintech) for 1 hour at room temperature. An enhanced chemiluminescence substrate kit (Millipore) was used for chemiluminescent detection of reactive signals with autoradiography films (Amersham, Little Chalfont, UK).

**Flow Cytometry**

Cell apoptosis was assessed by using an Annexin V-FITC Apoptosis Detection Kit (BD Biosciences, San Jose, CA, USA) according to the instructions. The cells were washed with PBS and resuspended in 200 μL binding buffer, stained with Annexin V-FITC for 15 minutes and Propidium Iodide for 5 minutes, and then subjected to flow cytometry analysis.

**Enzyme-Linked Immunosorbent Assay**

The serotonin concentrations in the tear fluid washings were determined with an ELISA serotonin kit (Enzo Life Sciences, Inc., Farmingdale, NY, USA). The IL-1β and TNF-α concentrations in the rat tear fluid washings were determined with ELISA kits for rat IL-1β (catalog No. ERL1B; ebioscience, San Diego, CA, USA) and rat TNF-α (catalog No. 88-7340-22; ebioscience) according to the manufacturer’s instructions. The IL-1β and TNF-α concentrations in the cell supernatants were determined with ELISA kits for human IL-1β (catalog No. KH0011; ebioscience), human TNF-α (catalog No. BM8203-5TEN; ebioscience), and human IL-10 (catalog No. 88-7106-70; ebioscience) according to the manufacturer’s instructions. The optical absorbance was measured at 450 nm with a microplate reader (Bio-Tek ELx800; Bio-Tek Instruments, Winooski, VT, USA), and the concentration of each sample was measured according to the instruction manual.

**Quantitative Real-Time RT-PCR Analysis**

Total RNA was extracted from the cells by using the TRI reagent according to the manufacturer’s protocol (Vazyme, Nanjing, China). cDNA was synthesized from the total RNA by using the Transcriptor First Strand cDNA Synthesis Kit (Roche, Indianapolis, IN, USA). The real-time PCR was performed in a 96-well optical plate with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), using the primers listed in the Table. The threshold cycle (Ct) values of the target genes were calculated with GAPDH normalization, and the transcriptional levels were analyzed with the 2^(-ΔΔCt) method.

**Statistical Analysis**

The statistical analysis was performed with SPSS 16.0.0 (SPSS, Chicago, IL, USA). All summary data are reported as the mean ± SD. Comparisons between groups were performed with an unpaired, 2-tailed Student’s t-test. P < 0.05 was considered statistically significant.

**RESULTS**

**SSRI Treatment Is Closely Related to Depression-Associated Dry Eye Disease**

No significant differences were found in the baseline data between the SSRI group and the controls (P > 0.05) (Fig. 1A). After 90 days of SSRI treatment, a slight improvement was observed in depression, and significant differences were found in the SAS, OSPI, CFS, and BUT results between the two groups (P < 0.05) (Fig. 1A). No differences were observed in the SBS, meibum quality, meibomian gland expressibility, and BUT results between the two groups (P > 0.05) (Fig. 1A). These findings suggest that SSRI may aggravate depression-associated DED.

**SSRI Treatment Causes Increased Tear Serotonin Levels, a Serious Inflammatory Response and Cell Apoptosis on the Ocular Surface**

To explore the role of SSRIs in depression-associated DED, we analyzed the serotonin and inflammatory cytokine levels in tears. The ELISA assay showed that patients with SSRI treatment had higher tear serotonin levels than the controls (2.66 ± 0.63 ng/mL versus 1.18 ± 0.33 ng/mL, P < 0.01) (Fig. 1B). The protein chip analysis revealed more inflammatory cytokines following SSRI use, based on examination of 507 proteins, including TLR4, IL-6, and IL-10 (Figs. 1C, 1D). TLR4 protein expressions were increased significantly (P < 0.01) (Figs. 1E, 1F). In addition, we found slightly increased inflammatory cytokines and proapoptotic genes in conjuncti-
val epithelial cells from the control group (placebo treatment) compared to those in the normal group ($P < 0.05$). Taken together, these results suggest that SSRI treatment aggravates depression-associated DED by exacerbating the inflammatory response and cell apoptosis on the ocular surface.

**Depression Can Decrease Tear Secretion and Increase IL-1β and TNF-α Production in Rats**

To confirm the role of depression in DED, we established a rat depression model. In the experiment, the rats were subjected to different mild stressors for 28 days. From the behavioral observations, the sucrose preference test, the forced swim test, and the open-field test, we confirmed that the rat depression model was established successfully (Figs. 2A–C). Tear secretion was significantly decreased in the depression group rats ($P < 0.01$), whereas no difference was found in tear secretion between the groups ($P > 0.05$) (Figs. 3A–C). Corneal staining also showed that the staining was diffuse and progressively intense in the SSRI group (Fig. 3D). ELISA assays demonstrated that the IL-1α and TNF-α levels were significantly increased in the SSRI group ($P < 0.01$) (Figs. 3E–G). In addition, we applied an SSRI to normal rats for 3 or 6 weeks and found that the 5-HT, IL-1α, and TNF-α levels were significantly increased in the SSRI group ($P < 0.01$) (Figs. 3H, 3I). These results were consistent with the findings of the depression model experiment.

**TABLE.** Primers Used in This Study

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<td>r-GAPDH</td>
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The corneal staining scores increased gradually together with the tear serotonin levels in the SSRI group ($P < 0.01$), whereas no difference was found in tear secretion between the groups ($P > 0.05$) (Figs. 3A–C). Corneal staining also showed that the staining was diffuse and progressively intense in the SSRI group (Fig. 3D). The qRT-PCR, immunohistochemical staining, and ELISA assays demonstrated that the IL-1β, TNF-α, and TLR4 levels were significantly increased in the SSRI group ($P < 0.01$) (Figs. 3E–G). In addition, we applied an SSRI to normal rats for 3 or 6 weeks and found that the 5-HT, IL-1β, and TNF-α content in the tears increased similarly ($P < 0.01$) (Figs. 3H, 3I). Together, these results reveal that SSRIs play a role in progressive damage of the corneal epithelium and DED via increasing the tear serotonin levels.

**Serotonin Can Induce Corneal Epithelial Cell Inflammation and Apoptosis**

To further explore the role of serotonin in the corneal epithelium, we cultured corneal epithelial cells to assay the effect of serotonin level variation on cell inflammation and apoptosis. Indeed, TLR4, which is a key mediator of proinflammatory responses, gradually increased in accordance with serotonin. Then, the qRT-PCR assay showed that the inflammatory cytokine IL1β, IL6, IL10, and TNFα levels increased with the serotonin level. The inflammatory factors were nearly maximized when serotonin was added to the cell supernatant at concentrations of 25 ng/mL or higher ($P < 0.01$) (Fig. 4A). However, the TLR2 expression level exhibited no obvious change ($P > 0.05$) (Fig. 4A). These results were consistent with the findings of the depression model experiment.
verified by the Western blot assay (Figs. 4B, 4C). Similarly, the IL-1β, IL-10, and TNF-α content in the cell supernatants gradually increased in the presence of serotonin (P < 0.01) (Fig. 4D). Moreover, compared with those of the control, expression of the proapoptotic protein BAX, BAD, and AIF increased in the serotonin-treated corneal epithelial cells (P < 0.01) (Figs. 4E, 4F). The flow cytometry results also showed that serotonin treatment led to an increase in apoptosis in corneal epithelial cells (Fig. 4G). Taken together, these observations indicated that serotonin induced cell inflammation and apoptosis in corneal epithelial cells and aggravated DED.

**Serotonin Receptors Are Indispensable for Serotonin-Induced Corneal Epithelial Cell Inflammation and Apoptosis**

To confirm the role of serotonin in cell inflammation and apoptosis of the corneal epithelium, we treated corneal...
epithelial cells with a specific inhibitor of serotonin. First, we detected the types and amounts of serotonin receptors (HTRs) in the corneal epithelial cells. Compared with cytokeratin 19, which is an epithelial cell biomarker, HTR1B, 1D, 2B, 2C, 3A, 4, and 5A were abundant in the corneal epithelial cells (Fig. 5A). Accordingly, we chose asenapine maleate (AM), which is a high-affinity and broad-spectrum antagonist of HTRs, to inhibit the efficacy of serotonin in the corneal epithelial cells. Then, the Western blot assays showed that the expression level changes of the proinflammatory response element TLR4, the inflammatory cytokines IL-6 and IL-10, and the proapoptotic proteins BAX, BAD, and AIF were all reversed in the AM-treated corneal epithelial cells (Figs. 5B, 5C). Similarly, we observed that the increase in the IL-1β, IL-10, and TNF-α content in the cell supernatants was reversed by asenapine maleate (Fig. 5D). In addition, decreased cell apoptosis was observed after asenapine maleate treatment of cells with increased serotonin levels by flow cytometry (Fig. 5E). These findings indicate that serotonin in combination with HTRs in corneal epithelial cells is essential for aggravating DED.

**Serotonin Induces Cell Inflammation and Apoptosis by Activating the NF-κB Pathway**

In an effort to identify the underlying mechanisms by which serotonin induced cell inflammation and apoptosis in corneal epithelial cells, we reviewed the related literature and found that the NF-κB pathway was closely related to serotonin and...
Figure 3. An SSRI increases the tear serotonin levels and aggravates dry eye disease in rats with depression. (A) ELISA analysis showing the tear serotonin levels in the SSRI and depression groups at 0, 3, and 6 weeks. (B-D) The parameters of the SIT and corneal fluorescence staining assays in the SSRI and depression groups at 0, 3, and 6 weeks. (E) qRT-PCR analysis of the corneal Tlr2, Tlr4, Il1β, and Tnfα mRNA levels in the SSRI and depression groups at 0, 3, and 6 weeks. (F) Immunohistochemistry staining showing TLR4, IL-1β, and TNF-α expression in the corneal tissues from the SSRI and depression groups at 0 and 6 weeks. (G) ELISA analysis showing the tear IL-1β and TNF-α levels in the SSRI and depression groups at 0, 3, and 6 weeks. (H, I) ELISA analysis showing the tear serotonin, IL-1β, and TNF-α levels in the SSRI and normal groups at 0, 3, and 6 weeks. *P < 0.05, **P < 0.01.
FIGURE 4. Serotonin can induce corneal epithelial cell inflammation and apoptosis. (A) qRT-PCR analysis showing the mRNA levels of the inflammatory cytokines TLR4, TLR2, IL1β, IL6, IL10, and TNFα with the serotonin level. (B, C) Western blot analysis showing the protein levels in the growth plate of TLR4, TLR2, IL-1β, IL-6, IL-10, and TNF-α with the serotonin level. (D) ELISA analysis of the related IL-1β, IL-10, and TNF-α content in the cell supernatants in cells with increased serotonin levels. (E, F) Western blot analysis showing the protein levels in the growth plate of the proapoptotic proteins BAX, BAD, and AIF with the serotonin level. (E) Flow cytometry showing apoptosis in corneal epithelial cells with serotonin treatment. *P < 0.05, **P < 0.01.
had regulatory effects on cell inflammation and apoptosis. Interestingly, we found that the p65 phosphorylation level (a subunit of NF-κB) was significantly increased, with a significant decrease in IκBα protein abundance, with serotonin treatment ($P < 0.01$) (Figs. 6A, 6B). These results verified that the NF-κB pathway was observably activated in the serotonin-treated cells. To determine whether the NF-κB pathway was involved in serotonin-induced cell inflammation and apoptosis, we used a specific inhibitor of NF-κB signaling (JSH-23) in the serotonin-treated cells. The Western blot assay showed that the expression level changes in P-p65, IκBα, the proinflammatory response element TLR4, inflammatory cytokines IL-6 and IL-10, and proapoptotic proteins BAX, BAD, and AIF in asenapine maleate–treated corneal epithelial cells. (D) ELISA analysis of the related IL-1β, IL-10, and TNF-α content in the supernatants of cells with accumulated serotonin treated with asenapine maleate. (G) Flow cytometry showing apoptosis in corneal epithelial cells with serotonin treated with asenapine maleate. *$P < 0.05$, **$P < 0.01$.}

**DISCUSSION**

DED is a multifactorial disease with higher morbidity in the depressed population. Patients with psychiatric disorders have more serious dry eye symptoms, including dryness, burning, and itching. Furthermore, DED patients also have a higher incidence of depression (odds ratio, 2.11) and psychosis (odds ratio, 1.87). Antianxiety and antidepressant medications are
associated with DED or dry eye symptoms. Here, we found that depression and SSRIs were important risk factors of DED. One vital side effect of SSRIs is aggravating depression-associated DED by activating the NF-κB pathway. In this study, our findings showed that depression can decrease tear secretion and increase IL-1β and TNF-α production in rats. There is a close association between DED and depressive symptoms. This is consistent with the conclusion of Hallak et al., who report an association between the clinical diagnosis of DED and depression status, even after controlling for psychiatric medications. As 5-HT in tears is not increased in dry eye–related depression, it is different from SSRI-related dry eye. Although the mechanism of how DED and depression interrelate has not been fully elucidated, we have found common pathophysiology in the two diseases. There are common risk factors including female sex and menopause, suggesting the involvement of sex hormones in both diseases. In addition, the anti-inflammatory potential of ω-3 polyunsaturated fatty acids (PUFAs) has been shown to be helpful in alleviating dry eye signs and symptoms.

**FIGURE 6.** Serotonin induces cell inflammation and apoptosis by activating the NF-κB pathway. (A, B) Western blot analysis showing the p65 phosphorylation level and the IκBα protein level with the serotonin level. (C, D) Western blot analysis showing the P-p65, IκBα, proinflammatory response element TLR4, inflammatory cytokine IL-1β, and proapoptotic protein BAX, BAD, and AIF protein levels in JSH-23-treated corneal epithelial cells. (E) ELISA analysis of the related IL-1β, IL-10, and TNF-α content in the supernatants of cells treated with the NF-κB signaling inhibitor. (F) Flow cytometry showing apoptosis in corneal epithelial cells with accumulated serotonin following JSH-23 treatment. *P < 0.05, **P < 0.01.
ingly, a study\textsuperscript{26} has reported decreased levels of PUFAs in patients with depression. An increased $\omega$-6 to $\omega$-3 ratio promotes the production of proinflammatory cytokines including the interleukins IL-1 and IL-6, and the tumor necrosis factor TNF-$\alpha$. These cytokines provoke ocular surface inflammation in DED and produce negative moods by affecting neurotransmissions and signal transductions.\textsuperscript{27} Overall, depression-associated dry eye should be given much more attention.

DED caused by SSRIs has specific pathologic features. In this study, we found that SSRIs could aggravate DED by inducing serious inflammation and cell apoptosis in corneal epithelial cells rather than by significantly decreasing tear secretion. A higher tear serotonin level is the main cause of the serious inflammation and apoptosis of corneal epithelial cells. Some reports\textsuperscript{11} have also suggested that DED patients have higher tear serotonin levels, but the results have been partial and discontinuous. 5-HT acts through 5-HTRs to activate NF-$\kappa$B signaling.\textsuperscript{14} Activated NF-$\kappa$B increases transcription of downstream genes and induces a rapid inflammatory response and cell apoptosis. Serotonin receptors (HTRs), especially HTR1B, 1D, 2B, 2C, 3A, 4, and 5A, are abundant in corneal epithelial cells (Fig. 5A). The results systematically verified that binding of serotonin to HTRs in corneal epithelial cells and activation of the NF-$\kappa$B pathway were important etiological factors of DED. This result suggests that SSRIs can severely impair the ocular surface.

Serotonin regulation of inflammation and cell apoptosis on the ocular surface aggravates depression-associated DED. In this report, we verified that asenapine maleate can improve DED caused by SSRIs. We found that JSH-23, which is a specific NF-$\kappa$B signaling inhibitor, partially rescued the phenotype in corneal epithelial cells. In the NF-$\kappa$B signaling pathway, proinflammatory cytokines, lipopolysaccharide, and other molecules can activate the IxB kinase (IKK) complex. Next, IKK$\beta$ phosphorylates IxBz, targeting IxBz for ubiquitination and proteasomal degradation. Thereby, IxBz abundance is reduced, which can be used as a biomarker for NF-$\kappa$B signaling. With the p65 nuclear localization signal no longer occluded by IxBz, NF-$\kappa$B/Rel (p50/p65) translocates into the nucleus where it binds to DNA sites to activate hundreds of antimicrobial and proinflammatory genes. Here, we found that the p65 phosphorylation level (a subunit of NF-$\kappa$B) was significantly increased, with an obvious decrease in IxBz protein, with serotonin treatment. NF-$\kappa$B is essential for activating TLR4 downstream signaling and innate immunity.\textsuperscript{28,29} TLR4 initiates signaling through cleavage of the NF-$\kappa$B complex. Cleaved NF-$\kappa$B translocates to the nucleus and activates the transcription of downstream genes. Here, we found that TLR4 was increased. We speculated that NF-$\kappa$B might promote TLR4 transcription via direct binding to the promoter. Increased TLR4 can further activate downstream signaling and in turn cleave more NF-$\kappa$B. The positive feedback caused by serotonin increases the inflammatory cytokine and proapoptotic protein levels, resulting in a more serious inflammatory response and cell apoptosis. Taken together, our results indicate that the 5-HT/NF-$\kappa$B axis is required for depression-associated DED.

Reports\textsuperscript{30–32} have suggested that biological molecules represent a potential therapeutic strategy for DED. These molecules have been thoroughly investigated with animal models. Researchers have verified that the TNF-$\alpha$ blocker etanercept can decrease inflammation in the corneas of mice with experimentally induced DED.\textsuperscript{33} Owing to its complex

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**Figure 7.** The model of how serotonin induces cell inflammation and apoptosis by activating the NF-$\kappa$B pathway in corneal epithelial cells.
etiology, developing an effective strategy to remedy the depression-associated DED caused by SSRIs is difficult. Here, we used a specific inhibitor for serotonin in corneal epithelial cells, which improved the inflammatory response and cell apoptosis but probably affected the pharmacologic effect for depression. JSH-23, which is a specific NF-κB signaling inhibitor, has been reported to reduce inflammation. The effect of supplemental JSH-23 can be attributed to its effect on neuroinflammation and oxidative stress. In our experiment, we found that JSH-23 supplementation significantly improved the inflammatory response and cell apoptosis in corneal epithelial cells. Importantly, it had no obvious influence on the pharmacologic effect of the SSRIs. These results suggest that JSH-23 may be a potential candidate in a novel therapeutic strategy for preventing depression-associated DED.

Our data demonstrated that SSRIs can induce ocular surface inflammation and aggravate depression-associated DED by activating the NF-κB pathway. Importantly, the DED caused by SSRIs can be rescued by a specific inhibitor of NF-κB signaling. These findings provide new insights into the pathogenesis of DED and present a novel therapeutic strategy for depression-associated DED.

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