Original Article

Protective effects of the novel adenosine derivative WS0701 in a mouse model of posttraumatic stress disorder

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Aim: To investigate the effects of the novel N6-substituted adenosine derivative {(2R,3S,4R,5R)-3,4-dihydroxy-5-[6-[(4-hydroxy-3-methoxybenzyl)amino]-9H-purin-9-yl]tetrahydrofuran-2-yl} methyl decanoate (WS0701) on stress-induced excessive fear, anxiety, and cognitive deficits in a mouse model of posttraumatic stress disorder (PTSD).
Methods: Male mice underwent a conditioned foot shock and single prolonged stress procedure to induce PTSD. Contextual/cued fear, elevated plus-maze, open field and novel object recognition tests were conduced to assess PTSD-like behaviors. From d 1, the mice were orally administered WS0701 (7.5, 15, or 30 mg·kg^{-1}·d^{-1}) or paroxetine (10 mg·kg^{-1}·d^{-1}) for two weeks. Apoptosis of hippocampal neurons was detected using flow cytometry and TUNEL staining, and expression of Bcl-2 and Bax in the hippocampus was measured with Western boltting and qPCR assays.
Results: WS0701 administration significantly alleviated fear, anxious behaviors and memory deficits in the mouse model of PTSD. Furthermore, WS0701 administration significantly reduced the stress-induced apoptosis of hippocampal neurons, and increased the Bcl-2/Bax ratio in the hippocampus. The positive control drug paroxetine exerted similar effects on PTSD-like behaviors and hippocampal neuron apoptosis in the mouse model of PTSD, which were comparable to those caused by the high dose of WS0701.
Conclusion: WS0701 effectively mitigates stress-induced PTSD-like behaviors in mice, partly via inhibition of neuronal apoptosis in the hippocampus.

Keywords: posttraumatic stress disorder; WS0701; adenosine; AMG-1; paroxetine; fear; anxiety; memory deficits; hippocampus; apoptosis; Bcl-2; Bax

Introduction

Individuals exposed to life-threatening trauma are at risk for developing post-traumatic disorder (PTSD). People who develop PTSD respond to a traumatic experience with intense fear, helplessness or horror and may endure chronic psychological distress due to repeatedly reliving their trauma through intrusive flashback memories[1]. This re-experiencing of the trauma can foster the development of debilitating symptoms, such as persistent anxiety, exaggerated startle responses and cognitive impairments[2].

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These symptoms correlate with the dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis, the dysregulation of several neurotransmitter systems[3] and functional or structural changes in several brain regions[4]. The hippocampus is one of the most important regions for the development of PTSD and is involved in explicit memory processes and the encoding of context during fear conditioning. The hippocampus also appears to interact with the amygdala during the encoding of emotional memories, a process that is highly relevant to the study of trauma and PTSD. In animals, reduced hippocampal volumes and memory impairments can result from extreme stressors, high levels of stress-related hormones, and abnormal hippocampal function[5]. The currently used treatments for PTSD include selective serotonin reuptake

inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), sedatives, adrenergic blockers, monoamine oxidase inhibitors (MAOIs), atypical anti-psychotics, and anti-convulsants[6]. Because they have fewer side effects and are better tolerated, SSRIs are used as the first-line treatment for PTSD. Paroxetine and sertraline are the only Food and Drug Administration (FDA)-approved pharmacotherapies for PTSD, but these medications are not ideal. Thus, the development of new drugs for the treatment of PTSD is needed.

A growing body of evidence suggests that the adenosine system can have multiple effects such as anticonvulsant, anxiolytic, and sleep-inducing effects, and the adenosine system can inhibit the discharges of amygdala and locus coeruleus and have neuroprotective effects in some conditions. Adenosine-related drugs have been used for the treatment of anxiety disorder, panic disorder, schizophrenia, epilepsy, and drug addiction[7]. AMG-1 is an N6 substituted adenosine derivative extracted from *Armillaria mellea*, which is a traditional Chinese medicine. Previous research has shown that AMG-1 has neuroprotective[8] and anticonvulsant effects[9] that are related to reductions in cellular energy metabolism[10] and glutamate release[11] and inhibition of increases in intracellular calcium[12]. {(2R,3S,4R,5R)-3,4-dihydroxy-5-[6-[(4-hydroxy-3-methoxybenzyl)amino]-9H-purin-9-yl]tetrahydrofuran-2-yl} methyl decanoate (WS0701), the structure of which has been confirmed by ^{1}H-NMR and ESI-MS[13] (Figure 1), is a modified form of AMG-1 that was created to increase the sedative effect of AMG-1.
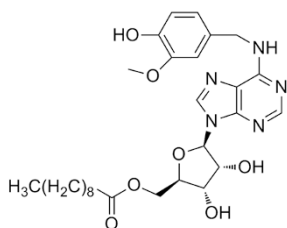

Figure 1. The chemical structure of WS0701.

PTSD is a severe anxiety disorder, but currently there is little evidence about the effects of adenosine or its derivatives in the regulation of PTSD in animal models. Therefore, we were interested in whether the adenosine derivative WS0701 would have effects on the behavioral changes induced in the PTSD mouse model. Furthermore, we explored the protective effects of WS0701 against aversive stress-induced neuronal apoptosis in the hippocampus.

Materials and methods
Animals
Male KunMing mice were obtained from the CoLab Biotechnology Ltd Laboratory Animal Center (Beijing, China, certificate N̲o̲ SCXK2009-2007). The mice were clean animals and initially weighed 12±2 g. The mice were housed 6 per cage in

a controlled environment with a 12 h light-dark cycle (lights on 7:00–19:00) at 23±2 °C and 45%±10% humidity. Food and water were available *ad libitum*. Animals received 5 d of habituation to the housing conditions before the stress handling. During the 5-d habituation period, the mice were handled once daily. Handling consisted of holding the animal with gloved hands for 1 min. All animal experiments were performed during the light phase from 9:00 to 17:00. All animal treatments were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experiments were performed under the approval of Experimental Animal Administration Committee of Peking Union Medical College.

Drugs and administration
The drugs used were paroxetine (Seroxat® tablet; GlaxoSmithKline KK, Tianjin, China) and WS0701 (purity over 98% as determined by HPLC and NMR; provided by the Institute of Materia Medica at Peking Union Medical College, Beijing, China). Both paroxetine and WS0701 were dissolved in 20% (*w/v*) hydroxypropyl-β-cyclodextrin (HP-β-CD, AR, Sinopharm Chemical Reagent Co, Ltd, Beijing, China) and then diluted to the proper concentration. Animals were randomized and placed into one of the following 6 groups: the Control, CF+SPS (conditioned foot shock and single prolonged stress), paroxetine (10 mg/kg), or one of three WS0701 (7.5, 15, and 30 mg/kg) groups. The drugs or vehicle were administered intragastrically once daily 30 min before the experiment from d 1 to d 14. Behavioral assessments were conducted on the corresponding days and video-recorded for off-line scor-
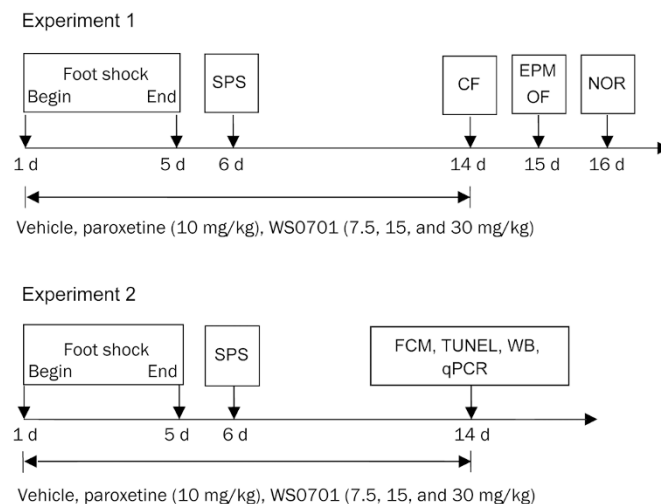

Figure 2. Experimental schedule. Experiment 1: conditioned foot shock (CF) sessions were continued for 5 d, and SPS occurred on the 6th d. The behavioral tests were conducted on the corresponding days. Experiment 2: the procedures for establishing the model were identical to those of Experiment 1. Flow cytometry (FCM), terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL), Western blotting (UB), and qPCR were performed on the 14th d.

ing (shown in Figure 2). All behavioral tests were conducted in a sound-proof testing room with constant illumination to which the animals were habituated for 2 h in their home cages before the initiation of the experiments. All behavioral scoring was performed by two blinded, trained observers.

Study procedures

Experiment 1

We conducted different behavioral tests to study the effects of chronic WS0701 treatment on fear responses, anxiety levels and memory deficits in the stressed mice. The retention of contextual fear was assessed by observing freezing behavior after re-exposure to trauma-associated cues. Anxiety levels were tested using the elevated plus maze (EPM) and open field (OF) test, and memory impairment was assessed using novel object recognition (NOR); n=12 animals per group (Figure 2).

Experiment 2

To investigate the effects of WS0701 on apoptosis in the hippocampus, brains were collected under anesthesia on d 14 after the drug treatment for flow cytometry (FCM, n=5), terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL, n=3), Western blotting (n=7) or qPCR (n=3) (Figure 2).

Conditioned foot shock and single prolonged stress procedure

For training, we used a Plexiglass chamber (15 cm×15 cm×40 cm) with a stainless steel grid floor. Mice were placed into the shock chamber; after a 60-s adaptation period, a bright light was turned on in the chamber. After 10 s, the mice received inescapable electric foot shocks (intensity: 1 mA, duration: 5 s) delivered through the grid floor by an isolated shock generator (YLS-9A, Yiyan Co, Ltd, Ji-nan, China). Control animals underwent the same procedure but were not subjected to electric foot shocks. This procedure was repeated for 5 consecutive days. A single prolonged stress (SPS) procedure was carried out on the 6th d. Mice were immobilized for 2 h in a Plexiglas mouse holder device, followed immediately by a 20-min forced swimming test in 24±1 °C water. The forced swim was carried out in a plastic tub (20 cm diameter, 30 cm height) that was two-thirds full of water. Following a 15-min recuperation period, the mice were exposed to ether until they lost consciousness, and they were then returned to their home cages[14, 15].

Conditioned fear test

To study contextual fear, the mice were re-exposed to the shock chamber for 5 min and received a further shock application without light presentation. To test for cued fear, the mice were placed into the neutral test chamber. After 30 s, a light was presented for 5 min, and the mice were placed back in their home cages after the tests. "Freezing" behavior was defined as sustained immobility (excluding respiration movements). Fear was quantified as the time (in seconds) that each mouse spent "freezing" [14].

Elevated plus maze test

The EPM apparatus consists of two open and two enclosed arms (30 cm×5 cm) that face each other and are attached to a center platform (5 cm×5 cm) that is elevated 50 cm off the floor. Each mouse was placed on the center platform and was allowed to explore the maze for 5 min. The time spent in and the visits to the open and closed arms during the observation period were recorded, and the percentage of open arm (OA) entries and the time spent in the OAs were calculated[16].

Open field test

Each mouse was placed in the center of a cubic chamber (40 cm×40 cm×35 cm) with a black floor. The floor was divided into sixteen squares (4×4). The testing room was dimly illuminated with indirect white lighting. Each animal was placed in the chamber for 5 min at a fixed starting point. The distance traveled in the central area and the time spent in the central area (defined as the mouse being in the central squares) were recorded using a behavioral analysis system (Dig-Behav, Jiliang Co, Shanghai, China)[17].

Novel object recognition test

This experiment consisted of three sessions: the habituation phase (5 min), the training phase (10 min), and the test phase (5 min). In the habituation phase, the mouse was placed into a wooden chamber (50 cm×50 cm×40 cm) for 5 min without an object. Then, during the training phase, two identical objects were placed near the corners of the chamber (10 cm from each adjacent wall). Each mouse was placed in the center of the floor and allowed to explore both objects for 10 min. Exploration was defined as the mouse positioning its nose toward the object at a distance of less than 2 cm. The time spent sitting or resting against the object was not recorded. The test phase occurred four hours after the training phase. In the test phase, the mouse was placed into the box, which contained one familiar object and one novel object, for 5 min. The exploration time for each object for each animal was collected manually by an experimenter blind to the treatment. The recognition rate (%) was defined as the novel object exploration time/total exploration time×100[18].

Analysis of apoptosis by flow cytometry

The mice were anesthetized with 10% (*w/v*) chloral hydrate and sacrificed by decapitation, and the hippocampus was dissected. Each sample was placed into cold PBS and made into a single-cell suspension, and the cell concentration was adjusted to $1×10^4$/mL. Next, 1 mL suspension was taken out and centrifuged at 800 rounds per minute at 4 °C for 5 min, and the supernatant was discarded. Then, 1 mL cold PBS was added and shaken gently to resuspend the cells. Centrifugation was repeated three times to obtain purified pellets. Cell pellets were resuspended in 500 µL binding buffer and then incubated with 5 µL Annexin V-FITC and 5 µL propidium iodide while being shielded from light at room temperature for 15 min. Finally, apoptotic cells were analyzed with a FACSCalibur® flow cytometer (BD Biosciences, Burlington, MA, USA).

Approximately 1×10^4 cells from each sample were analyzed[19].

Immunohistochemical staining

After anesthesia with 10% (*w/v*) chloral hydrate, the mice were perfused with 4% paraformaldehyde (PFA)/PBS (0.01 mol/L), pH 7.2, and the brains were removed. Samples were dehydrated in alcohol and embedded in paraffin. Coronal sections of approximately 2 μm were prepared for morphological studies. Dewaxed sections were washed three times (5 min each) in 0.01 mol/L PBS and permeabilized in proteinase K for 10 min. Endogenous peroxidase was deactivated by 0.3% hydrogen peroxide. The sections were washed three more times and were then incubated with TdT at 37 °C for 1 h, followed by incubation with antibody at 37 °C for 1 h. After hematoxylin post-staining, the sections were stained with 3,3'-diaminobenzidine, mounted, and photographed under a light microscope. The Image-Pro Plus software (Media Cybernetics, Silver Spring, MA, USA) was used for image analysis. The TUNEL-positive cell rate was calculated as the number of TUNEL-positive cells/total cells×100. Three animals in each group were analyzed, and the mean TUNEL-positive cell rate was determined[20].

Western blotting analysis

Seven animals were analyzed in each group. Protein lysates were obtained by homogenization with lysis buffer. Protein content was determined using the BCA method. Fifty-microgram protein samples were separated on SDS-polyacrylamide gels and transferred onto PVDF membranes. Rabbit Bcl-2 antibody (1:1000) and rabbit Bax antibodies (1:1000) were used as the primary antibodies (Cell Signaling Technology, Inc, USA). HRP-conjugated anti-rabbit antibodies for Bcl-2 and Bax were used as secondary antibodies (1:2000, Santa Cruz Biotechnology, Inc, USA). Band detection was performed using an enhanced chemiluminescence detection system (ECL, Molecular Imager ChemiDoc XRS+ System, Bio-Rad, Hercules, CA, USA). To confirm equal protein amounts, the same blots were incubated with antibodies specific for β-actin (Santa Cruz Biotechnology; 1:2000). The band volumes and densities were measured using the Quantity One 1D analysis software (Bio-Rad, Hercules, CA, USA).

Quantification of hippocampal mRNA expression levels

Total RNA was extracted using TRIzol® reagent (Invitrogen Inc, CA, USA), following the manufacturer's instructions. RNA was quantified with optical density measurements at 260 and 280 nm. Reverse transcription was then performed on 1 μg of total RNA using the SuperScript First-strand Synthesis SuperMix (Invitrogen Inc, CA, USA). The reaction was incubated at 42 °C for 10 min and 95 °C for 2 min. Reverse transcription products were stored at -80 °C. qPCR was performed on the cDNA samples. The GAPDH gene was used to normalize the expression. qPCR was performed in a volume of 25 μL reaction mixture that contained 2 μL cDNA, 12.5 μL 2.5×TransStart® Green qPCR SuperMix, 0.5 μL 10 μmol/L forward primer, 0.5 μL 10 μmol/L reverse primer and DNase-free water using a qPCR Kit (TransGen Inc, CA, USA). The samples were heated to 95 °C for 30 s. The cycles were 95 °C for 5 s, 55 °C for 15 s and 72 °C for 10 s. Relative gene expression was determined using the CFX Manager® software (Bio-Rad, Inc, Hercules, CA, USA) according to the $2^{-\Delta\Delta Ct}$ method[21]. Additionally, derived melting curve analyses were performed to assess the specificity of the amplification reaction. The following primers were used:

Bcl-2 forward: 5'-ACCGTCGTGACTTGGCAGAG-3';

Bcl-2 reverse: 5'-GGTGTGCAGATGCCGGTTCA-3';

Bax forward: 5'-CGGCGAATTGGAGATGAACTG-3';

Bax reverse: 5'-GCAAAGTAGAAGAGGGCAACC-3';

GAPDH forward: 5'-TAAAGACCTCTATGCCAACA-CAGT-3'; and

GAPDH reverse: 5'-CACGATGGAGGGGCCGGACT-CATC-3'.

Statistical analysis

All statistical analyses were performed using SPSS (Statistical Product and Service Solutions) 20.0 (Chicago, IL, USA). The data are presented as the mean±SD. Statistical analysis included one way analyses of variance (ANOVAs) followed by Newmann-Keuls tests, when appropriate. For all tests, $P<0.05$ was considered significant.

Results

Chronic WS0701 treatment reduced freezing behavior

As shown in Figure 3, the contextual fear and cued fear of the CF+SPS group [$F_{(5,66)}=10.288$, $P<0.01$; $F_{(5,66)}=8.220$, $P<0.01$] were significantly increased compared with the control group, and WS0701 administration decreased contextual fear and cued fear compared to the CF+SPS group (15 mg/kg: $P<0.05$; 30 mg/kg: $P<0.05$).

Chronic WS0701 treatment decreased the animals' anxiety

Elevated plus maze test

As shown in Figure 4A and 4B, the OA entries and OA times of the CF+SPS group in the EPM were decreased compared to the control group [$F_{(5,66)}$ entries=42.996, $P<0.001$; $F_{(5,66)}$ time=20.931, $P<0.001$]. At doses of 7.5, 15, and 30 mg/kg, WS0701 significantly increased the number of OA entries and the OA time compared to the CF+SPS group ($P<0.05$).

Open field test

The open field test results showed that the time spent in the center area and the central traveling distances of the CF+SPS group were significantly decreased compared to the control group [$F_{(5,66)}$ time=10.908, $P<0.001$, $F_{(5,66)}$ distance=21.153, $P<0.001$]. The administration of WS0701 (15 and 30 mg/kg; $P<0.05$) increased the time spent in the central area and the traveling distance in the central area compared to the CF+SPS group (Figure 4C, 4D).

Chronic WS0701 treatment reduced memory impairments

The mice in the control group spent more time exploring the novel object than the familiar object during the test session
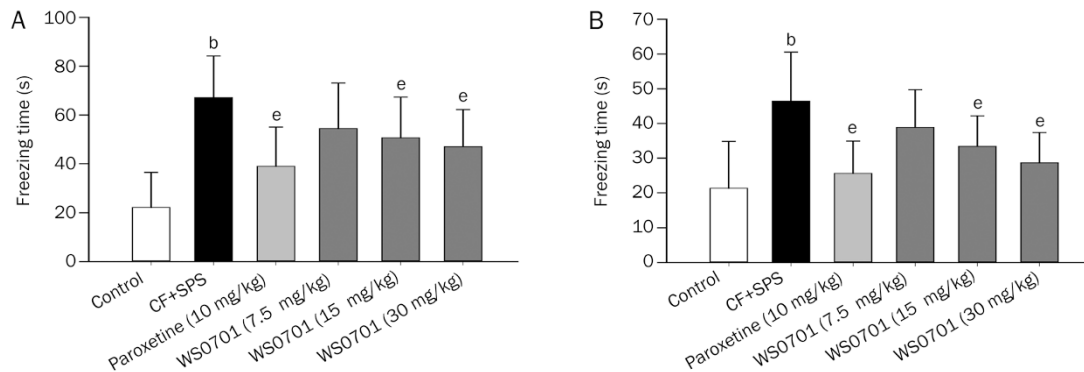
Figure 3. Chronic WS0701 treatment reduced freezing behavior in stressed mice. (A) Contextual fear test. (B) Cued fear test. Values are expressed as the mean±SD. [b]$P<0.05$ compared to the control group. [e]$P<0.05$ compared to the CF+SPS group.
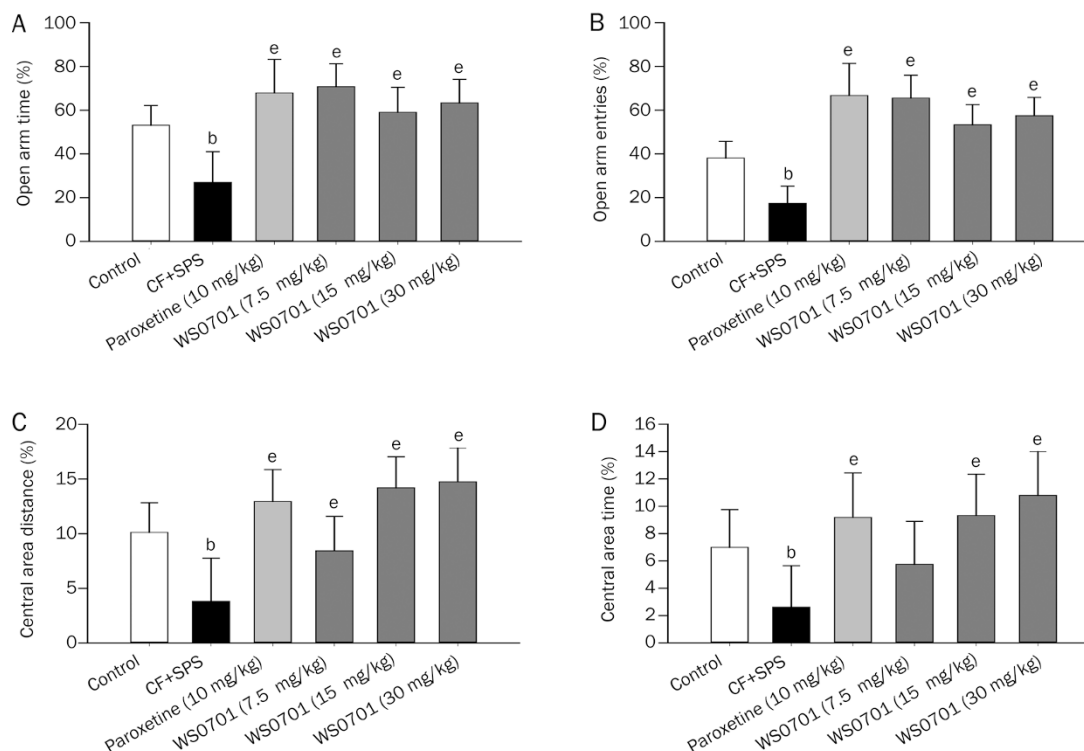

Figure 4. Chronic WS0701 treatment decreased the anxiety of stressed mice in the elevated-plus maze and open field tests. The EPM and OF tests were 5 min in duration. (A) Percentages of time spent in the open arms; (B) Percentages of entries into the open arms; (C) Percentages of travel distance in the central area; (D) Percentages of time spent in the central area. The values are expressed as the mean±SD. [b]$P<0.05$ compared to the control group. [e]$P<0.05$ compared to the CF+SPS group.

$[F_{(5,66)}=6.582, P<0.001]$. In contrast, the stressed mice spent significantly less time exploring the novel object ($P<0.05$), which indicated the presence of memory deficits in this group. Mice treated with WS0701 (15 mg/kg, $P<0.05$) spent more time exploring the novel object than the mice in the CF+SPS group (Figure 5).

Chronic WS0701 treatment reduced hippocampal apoptosis
Flow cytometry assay
As shown in Figure 6, we performed Annexin V-FITC/PI

double-labeled flow cytometry to assess early apoptosis in the hippocampus. The percentage of early apoptotic cells was increased in the stressed mice $[F_{(5,24)}=28.830, P<0.01]$. Compared with the CF+SPS group, paroxetine (10 mg/kg, $P<0.05$) and WS0701 (15 and 30 mg/kg, $P<0.05$) treatment significantly decreased apoptotic cell percentages.

TUNEL staining
TUNEL-positive cells were rarely detected in the hippocampal dentate gyri of the control group. In contrast, stress signifi-
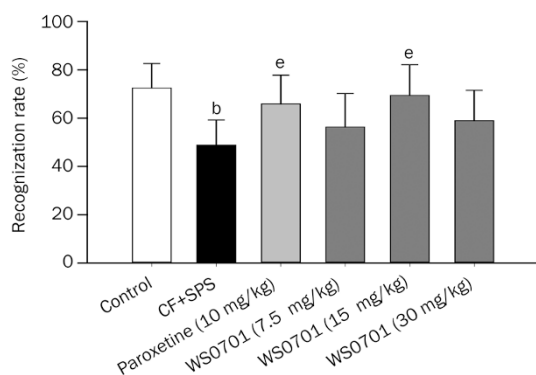
Figure 5. Chronic WS0701 treatment inhibited stress-induced memory impairments in the novel object recognition test. The values are expressed as the mean±SD. [b]$P<0.05$ compared to the control group. [e]$P<0.05$ compared to the CF+SPS group.

cantly promoted neuronal apoptosis compared to the control group [$F_{(5,12)}=17.220$, $P<0.01$]. WS0701 (15 and 30 mg/kg, $P<0.05$) and paroxetine (10 mg/kg, $P<0.05$) treatment signifi-

cantly reduced the numbers of apoptotic neurons in the dentate gyrus (Figure 7).

Chronic WS0701 treatment increased the Bcl-2/Bax ratio in the hippocampus

Compared to the control group, both Western blotting and qPCR results showed that the stress procedure increased the expression of Bax [$F_{Protein(5,36)}=10.724$, $F_{Gene(5,12)}=17.99$, $P<0.05$] and decreased the Bcl-2/Bax ratio in the hippocampus. The administration of paroxetine (10 mg/kg) or WS0701 (15 and 30 mg/kg) reduced the expression of Bax ($P<0.05$) and increased the Bcl-2/Bax ratio, which may be related to the anti-apoptotic effects of these drugs (Figure 8).

Discussion

The goal of the present study was to evaluate the long-term effects of the novel adenosine derivative WS0701 on fear, anxiety and memory impairment in a mouse model of PTSD. Animal models play an important role in the evaluation of novel pharmacotherapeutic agents for PTSD. Previous studies have reported on the fear conditioning model[22] and the single pro-
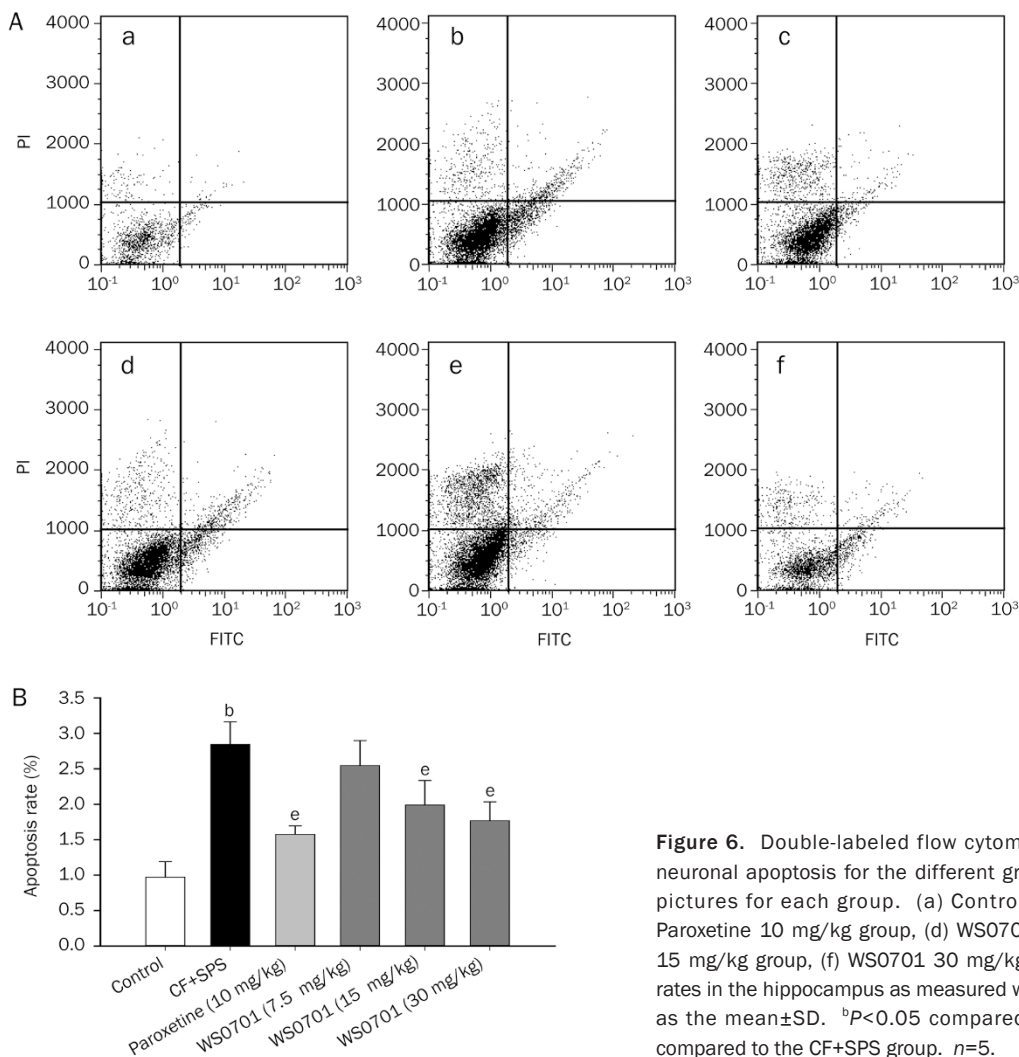

Figure 6. Double-labeled flow cytometric analyses of hippocampal neuronal apoptosis for the different groups. (A) shows flow cytometry pictures for each group. (a) Control group, (b) CF+SPS group, (c) Paroxetine 10 mg/kg group, (d) WS0701 7.5 mg/kg group, (e) WS0701 15 mg/kg group, (f) WS0701 30 mg/kg group. (B) shows the apoptosis rates in the hippocampus as measured with FCM. The data are expressed as the mean±SD. [b]$P<0.05$ compared to the control group, [e]$P<0.05$ compared to the CF+SPS group. $n=5$.
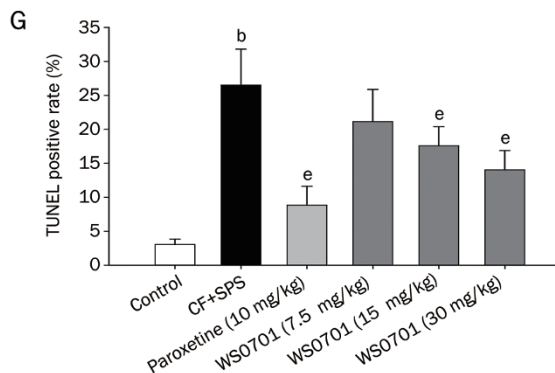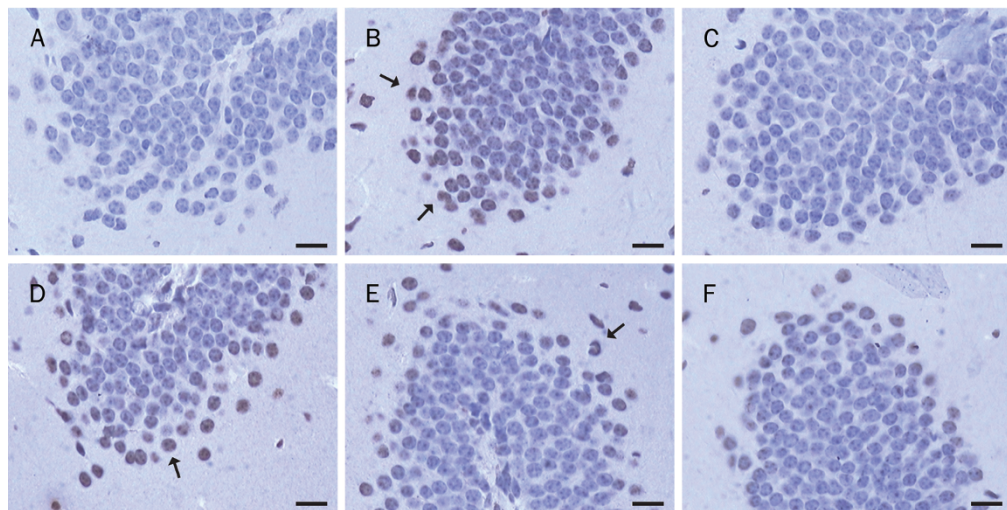
Figure 7. Representative TUNEL staining pictures from hippocampal dentate gyrus sections. The arrows indicate apoptotic neurons. The values are expressed as the mean±SD. [b]$P<0.05$ compared to the control group. [e]$P<0.05$ compared to the CF+SPS group. (A) Control group, (B) CF+SPS group, (C) Paroxetine 10 mg/kg group, (D) WS0701 7.5 mg/kg group, (E) WS0701 15 mg/kg group, (F) WS0701 30 mg/kg group, and (G) the TUNEL-positive cell rates for each group. Scale bar=10 μm, n=3.
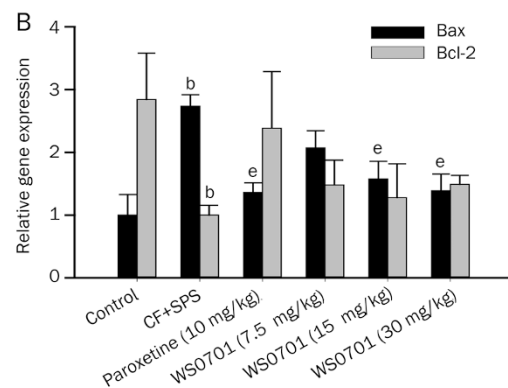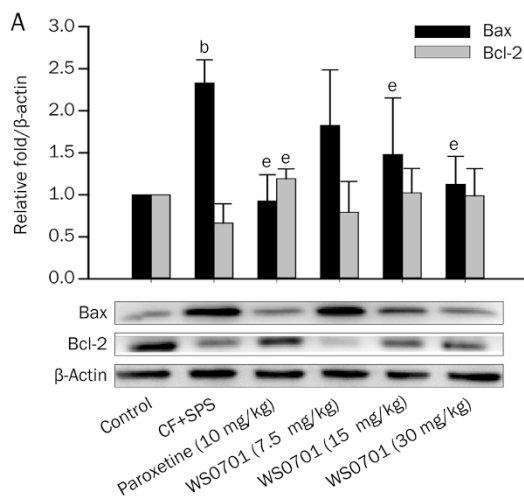

Figure 8. Chronic WS0701 treatment increased the Bcl-2/Bax ratios in the hippocampi of stressed mice. (A) Effect of WS0701 on Bax and Bcl-2 protein expressions. (B) Effect of WS0701 on Bax and Bcl-2 gene expressions. The values are expressed as the mean±SD. [b]$P<0.05$ compared to the control group. [e]$P<0.05$ compared to the CF+SPS group.

longed stress model[23]. These models mimic different symptoms of PTSD patients and have been widely used in PTSD research. Recent studies have reported that the combined application of the fear conditioning and SPS procedure can reproduce both the behavioral and endocrine changes of PTSD and that paroxetine has protective effects in this model; thus, we used paroxetine treatment as a positive control[14].

With this mouse model of PTSD, we demonstrated that the

animals submitted to the stressors not only exhibited specific avoidance of the context and cues associated with the aversive stress but also avoided entering the center squares and the open arms compared with the control mice. At doses of 15 and 30 mg/kg, WS0701 significantly decreased freezing behavior. In the elevated plus maze and open field tests, WS0701 increased the times spent in the open arms and the central area at all doses. Moreover, the stressed mice exhibited decreased novel object recognition abilities in a familiar environment, which indicates spatial memory deficits. At a dose of 15 mg/kg, WS0701 prevented the cognitive deficits induced by stress. Though the 30 mg/kg WS0701 group also exhibited some improvement relative to the control groups, this difference did not reach significance, which may, in part, be due to the stronger sedative effects of WS0701 at this dose. These results showed that WS0701 alleviated the fear responses of stressed animals in the context associated with the traumatic event and reduced anxiety levels and memory impairments induced by the aversive procedure.

PTSD often involves biochemical and molecular changes in the hippocampus. The results of the flow cytometry and TUNEL experiment indicated that apoptosis in the hippocampus significantly increased in the stressed mice, while the stressed mice treated with WS0701 (15 and 30 mg/kg) exhibited decreased levels of apoptosis. To reveal the possible mechanisms of action of WS0701, we used Western blotting and qPCR to examine the expression level of Bax and Bcl-2, which are important mediators of the mitochondria-mediated apoptosis pathway. The Bcl-2/Bax ratio is critical in determining whether apoptosis occurs. The down-regulation of the Bcl-2/Bax ratio may contribute to the reduced hippocampal volumes and impaired hippocampal function that are associated with PTSD. In this study, both the protein and gene expression levels of Bcl-2 and Bax were measured. Bcl-2/Bax ratios were significantly reduced in the hippocampi of the stressed mice, while paroxetine and WS0701 (15 and 30 mg/kg) significantly increased the Bcl-2/Bax ratios in the hippocampi of stressed mice.

PTSD is a severe anxiety disorder. Anxiolytics that are used clinically include benzodiazepines, SSRIs, opiate narcotics and non-benzodiazepine sedatives. SSRIs are currently the most widely used drugs for the treatment of PTSD. Paroxetine is approved by the FDA for the treatment of PTSD, and it has been reported that paroxetine can reduce PTSD symptoms in animal models[24]. Paroxetine possibly acts on monoamines in a nonspecific manner, increases neurotrophic factors and thus exerts neuroprotective effects in the hippocampus. Benzodiazepines are the classic anxiolytics and act on the GABA benzodiazepine receptor to produce sedative, hypnotic, anxiolytic and anticonvulsant effects. Although these properties suggest a useful role for benzodiazepines in PTSD, benzodiazepines have not been shown to treat PTSD effectively in clinical and animal studies[25]. Recent studies have revealed that the opiate analgesic morphine is effective in preventing PTSD in people experiencing physical injuries from traumatic events[26]. Morphine is an opioid receptor agonist, and the mechanism by which opioids can inhibit the development of PTSD may be due to pain relief and the blockade of fear memory consolidation; however, the use of opioids is limited by addiction and abuse problems[27].

Adenosine has been reported to have anxiolytic, sedative, hypnotic and anticonvulsant effects. It has also been revealed that the anxiolytic and neuroprotective effects of adenosine are related to the activation of the adenosine A_{2A} receptor[28, 29]. WS0701 was derived from AMG-1, which is structurally similar to adenosine. In our study, we found that WS0701 produced anxiolytic and neuroprotective effects that are similar to those of adenosine. Therefore, we infer that the mode of action of WS0701 may be related to the activation of the adenosine A_{2A} receptor, and this hypothesis will be studied in our future experiments.

Conclusion

In this study, the repeated administration of WS0701 exhibited a protective effect in a mouse model of PTSD. WS0701 administration reduced the anxiety, fear behaviors and memory deficits induced by aversive stressors, and its mechanism of action may be related to the inhibition of neuronal apoptosis in the hippocampus. We propose that WS0701 may have potential as a treatment for the prevention of PTSD and possibly other anxiety disorders after traumatic events. Further experiments are necessary to clarify the relationship between WS0701 and the regulation of emotionality during stressful conditions as well as the exact molecular mechanisms underlying the protective effects of WS0701.

Author contribution

Song WU synthesized and provided WS0701; Guan-hua DU directed the research and revised the manuscript; Zhong-lin HUANG performed the research and wrote the paper; Rui LIU, Xiao-yu BAI, Gang ZHAO, and Jun-ke SONG revised the manuscript.

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