European Review for Medical and Pharmacological Sciences 2022; 26: 1897-1905

Ciproxifan attenuates the memory impairment induced by lipopolysaccharide through modulation of cholinergic transmission in the mouse brain

V. MANI[1], M. ARFEEN[2], H.M. ALI[1,3], A.-M. HAFEZ ABDEL-MONEIM[4,5], M. ALDUBAYAN[1], M. DHANASEKARAN[6], A. ALHOWAIL[1]

[1]Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Buraydah, Kingdom of Saudi Arabia
[2]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Qassim University, Buraydah, Kingdom of Saudi Arabia
[3]Department of Biochemistry, Faculty of Medicine, Al-Azhar University, Assiut, Egypt
[4]Department of Physiology, Faculty of Medicine, Qassim University, Kingdom of Saudi Arabia
[5]Department of Physiology, Faculty of Medicine, Mansoura University, Egypt
[6]Department of Drug Discovery and Development, Harrison School of Pharmacy, Auburn University, Auburn, Alabama (AL), USA

Abstract. – OBJECTIVE: We investigated the protective effect of ciproxifan on lipopolysaccharide (LPS)-induced memory impairment by altering the cholinergic system in a mouse model.

MATERIALS AND METHODS: Groups of mice were given ciproxifan (1 or 3 mg/kg, p.o.) for 30 days. Neurotoxicity was induced with four doses of LPS (250 µg/kg, i.p.) from day-22 to day-25 of drug treatment in three groups. Then, mice were subjected to behavioral assessments using tests [elevated plus maze (EPM), novel object recognition (NOR), and Y-maze]. Also, brain tissues were collected for estimation of cholinergic transmission [acetylcholine (ACh) and acetylcholinesterase (AChE) levels].

RESULTS: Ciproxifan could rescue the memory impairment caused by LPS by shortening the transfer latency in the EPM test, increasing the time spent to explore a novel object and increasing the Discrimination Index in the NOR test and increasing the number of entries to the novel arm and duration of time spent in the novel arm in the Y-maze test. Ciproxifan increased the levels of ACh by decreasing AChE activity in LPS-treated mice.

CONCLUSIONS: Ciproxifan treatment can improve memory impairment in mice by increasing ACh levels and decreasing AChE levels.

Key Words:
Ciproxifan, Lipopolysaccharides, Memory impairment, Acetylcholine, Acetylcholinesterase.

Introduction

Alzheimer's disease (AD) is a neurological disease characterized by increased memory loss and reduced cognitive skills. Neuronal degeneration in AD is characterized principally by plaque aggregation and excessive production of beta-amyloid and neurofibrillary tangles[1]. AD and other types of dementia are regarded as a substantial healthcare burden worldwide. More than 50 million people worldwide suffer from dementia, with the figure expected to rise to approximately 152 million by 2050[2]. Memory and learning problems associated with dementia are thought to be caused by several factors: cholinergic insufficiency, oxidative stress, and neuroinflammation[3]. The decline in levels of the neurotransmitter acetylcholine (ACh) has been linked to neurodegenerative disorders and other aging-related memory problems. In addition, the enzyme acetylcholinesterase (AChE) catalyzes ACh breakdown in synaptic clefts affects cholinergic neurotransmission in the brain. As a result, medications that target the cholinergic system are a promising treatment option for AD patients[4].

Several histamine H_3 receptor (H3R) antagonists/inverse agonists are being tested in clinical studies for the treatment of minor cognitive deficits, AD, dementia, and other cognitive disorders[5]. These receptors are found mostly in brain regions, including the cerebral cortex and hippocampal formation, which are associated with cognition[6]. The H3R presents pre-synaptically and reduces the release of neurotransmitters, such as ACh, histamine, norepinephrine, and dopamine[7]. Increased levels of these neurotransmitters are thought to improve attention and memory, so H3R antagonists have been researched extensively and tested in behavioral models.

Corresponding Author: Vasudevan Mani, MD; e-mail: V.SAMY@qu.edu.sa

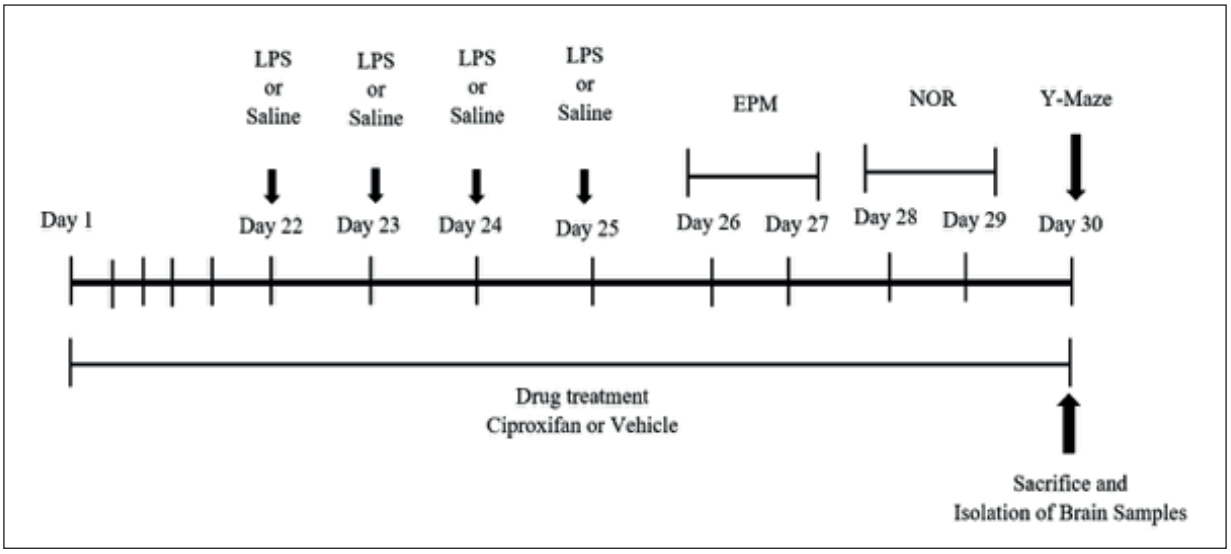

Figure 1. Timeline of drug administration, behavioral assessments, and isolation of brain samples.

Ciproxifan is a strong antagonist of H3Rs. It has shown anti-AD potential in transgenic mice with AD (B6.129-Tg(APPSw)40Btla/J) by increasing cognitive abilities, strengthening cholinergic transmission, improving antioxidant characteristics, and attenuating neuroinflammation[3]. Ciproxifan has been shown to reverse cognitive loss in a Tg2576 mouse model of early AD[8].

Lipopolysaccharide (LPS) is a component of the cell walls of Gram-negative bacteria. It causes neuronal toxicity in the brain by triggering neuroinflammatory responses, memory impairment, cholinergic insufficiency, and oxidative stress. LPS-induced memory impairment is linked primarily to neuroinflammation, which is associated with stimulation of microglial cells, production of proinflammatory mediators [e.g., tumor necrosis factor-α, interleukin (IL)-1α, IL-1β, and IL-6] in the brain, and degeneration of brain cholinergic neurons (notably in the hippocampus[9,10]). LPS can also inhibit ACh production in cholinergic neurons by decreasing the function of choline acetyltransferase and weakening cholinergic transmission between neurons[11]. The role of ciproxifan in inflammation-induced memory impairment is incompletely understood.

So, we investigated the neuroprotective ability of ciproxifan on LPS-induced memory deficit and cholinergic dysfunction in a mouse model.

Materials and Methods

Drugs and Chemicals

Ciproxifan maleate and LPS were obtained from MilliporeSigma (Burlington, MA, USA). Enzyme-Linked Immunosorbent Assay (ELISA) kits for ACh and AChE in mice were obtained from Cloud-Clone (Houston, TX, USA). All other chemicals and solvents were obtained from local suppliers and were of analytical grade.

Animals

The experimental protocol of our study was approved (2020-CP-7) by the Animal Ethical Committee of the College of Pharmacy and Deanship of Scientific Research of Qassim University (5601-pharmacy-2019-2-2-I). Animal maintenance followed the procedures set out in the *Guide for the Care and Use of Laboratory Animals* (US National Institutes of Health, Bethesda, MA, USA).

Twenty-four adult male ICR mice (8-12 weeks; 25-35 g) were collected from the animal facility of Qassim University (Buraydah, Saudi Arabia). Each polypropylene cage housed three mice, who had free access to food and water throughout the acclimatization period (5 days) and test period (30 days).

Experimental Design

Twenty-four mice were separated randomly into four groups of six and given vehicle or ciproxifan. Mice in the control group were treated with vehicle [physiologic (0.9%) saline: 10 ml/kg per day, p.o.] for 30 days and then received four doses of 0.9% saline (10 ml/kg, i.p.) between day-22 and day-25 of the treatment schedule (Figure 1). The LPS-induced group was treated with vehicle (0.9% saline: 10 ml/kg per day, p.o.) for 30 days and four doses of LPS (250 μg/kg, i.p.) from day-22 to day-25 of the treatment schedule. The LPS dose employed to

induce neurotoxicity in this mouse model was as described previously[12,13]. Two test groups (LPS+CIP1 and LPS+CIP3) were administered ciproxifan (1 and 3 mg/kg/day, respectively) *via* the oral route for 30 days and four doses of LPS (250 µg/kg, i.p.). The elevated plus maze (EPM) test, novel object recognition (NOR) test, and Y-maze test were conducted from day-26 to day-30 of the treatment schedule (Figure 1). All mice were killed on day-30 so that brain tissues could be harvested.

Survival and Bodyweight

Mice were examined from day-1 to day-30 and deaths were recorded. The bodyweight of each mouse was measured every 7 days (days 1, 8, 15, 22, 29).

EPM Test

The EPM was made of wood with four equal-sized arms of length 16 cm and width 5 cm and elevated 25 cm above the floor. Two arms were closed and surrounded by 12-cm high walls. The other two arms were open and on the opposite side.

Each mouse was placed at the end of the open arm, opposite the center platform, during the training session (day-26). The time taken (in seconds) by the mouse to move from the open arm to one of the closed arms with all its four feet was considered to be the transfer latency (TL). If the mouse did not enter the closed arm after 90 s, he was pushed gently into the closed arm and given another 2 min to explore the maze. After 24 h, the mouse was brought back to evaluate the TL: this session was recorded as "retention of learned-task memory"[14].

NOR Test

The NOR test was carried out in accordance with a method described previously with slight modification[15]. An open wooden box (80 × 60 × 40 cm) was utilized to carry out the NOR test. Two discriminating objects (familiar object: cylindrical box; novel object: rectangle box) of identical height and build were used to carry out the NOR test. Experiments were in three phases: habituation (day-28), familiarization, and testing (day-29). Each mouse was given 5 min to explore the box freely without objects during the habituation period. The familiarization phase (FP) was initiated after 24 h, and the mouse was allowed to explore the box, which now contained two similar familiar objects (FO1 and FO2), for 5 min. After 3 h, the test phase (TP) was undertaken. During the TP, the mouse was permitted to explore the box, which now contained two discriminating objects (a familiar object: FO1; novel object (NO): cylindrical box), for a maximum of 5 min. The time spent exploring the NO and FO1 was recorded. The "exploration time" refers to the amount of time an animal spends directing its nose to an object at a distance ≤2 cm and touching it with its nose[14,15]. During the TP phase, the capacity of a mouse to distinguish between FO1 and NO was calculated as the Discrimination Index (DI):

$$DI = N - F/N + E$$

where N is the exploration time of NO, and F is the exploration time of FO1.

Y-maze Test

The Y-maze test was carried out in accordance with a method described previously with slight modification[14,16]. The maze was constructed with three wooden arms at 120° (35 × 5 × 10 cm) between each other. A picture was fixed at each arm end to distinguish the arm. The test was conducted (day-30) with two sessions: training session (T1) and test session (T2). Among the three arms, arm 'A' was considered to be a novel arm, and arm 'B' was fixed and considered to be the starting arm. During T1, arm A was blocked, and the mouse was given 5 min to explore two familiar arms. T2 was carried out 3 h after T1. In T2, the mouse was given 5 min to explore all of the arms, including the novel arm (A). The number of entries to the novel arm and duration of time spent in the novel arm were recorded during T2. The "entry" of an animal is said to occurr if 85% of his/her body enters an area[16].

Biochemical Assays Using Brain Homogenates

On day-30, at the end of the Y-maze test, all mice were killed by cervical dislocation. The whole-brain of each mouse was extracted from his skull. Each brain sample was homogenized in ice-cold phosphate-buffered saline (4°C, pH 7.4) using a homogenizer. The collected homogenate was centrifuged at 4000 × g for 10 min at room temperature, transferred to a 4-ml vial, and stored at -80°C. The total protein content of the sample was quantified using the biuret colorimetric method (Crescent Diagnostics, Riyadh, Saudi Arabia). The sample was tested using ELISA kits for ACh and AChE according to manufacturer (Cloud-Clone) instructions. Absorbance was measured at 450 nm using a microplate reader (ELx800; BioTek Instruments, Winooski, VT, USA).

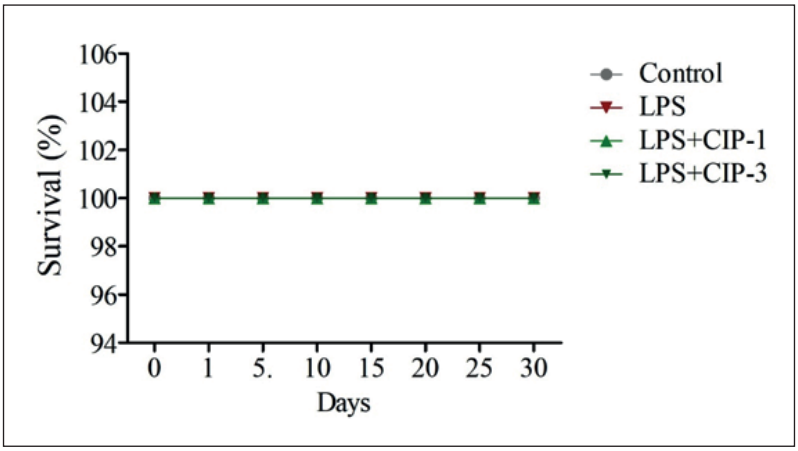

Figure 2. Effect of ciproxifan (CIP) and lipopolysaccharide (LPS) on mouse survival during the 30 days of the study.

Statistical Analyses

Results are the mean ± standard error of the mean (SEM). Comparisons between groups were analyzed using one-way ANOVA followed by the Tukey-Kramer post-hoc test to test the significance of differences between the two groups. Prism 9 (GraphPad, La Jolla, CA, USA) was employed for statistical analyses. $p < 0.05$ was considered as significant.

Results

Ciproxifan and LPS Do Not Alter Weight Loss

Figure 2 shows the effect of ciproxifan and LPS on the survival of mice during the treatment period. No mice died during 30 days of continuous treatment with ciproxifan (1 or 3 mg/kg) and 4 days of LPS (250 µg/kg) administration. A significant alteration in bodyweight with identical treatment on days 1, 8, 15, 22, and 29 was not observed as compared with that in the control group (Figure 3).

Ciproxifan Shortens the TL of LPS-Treated Mice in the EPM Test

A short TL in the EPM test is considered to denote improved memory capacity in rodents. Figure 4 highlights the effect of ciproxifan on the TL in LPS-treated mice. Differences for the TL [$F(3.20) = 35.32$, $p < 0.001$; one-way ANOVA] were observed upon comparison among all groups. Multiple post-hoc analyses of comparisons between

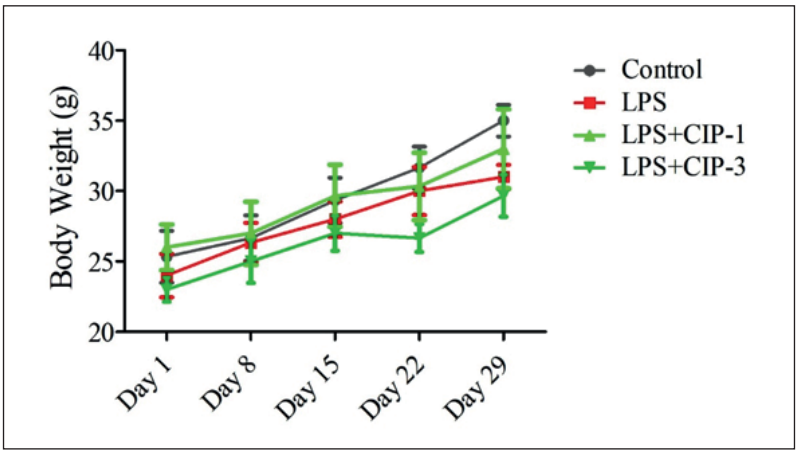

Figure 3. Effect of ciproxifan (CIP) and lipopolysaccharide (LPS) on the bodyweight of mice during the 30 days of the study. Results are the mean ± SEM (n = 6). One-way ANOVA [$F(3.20) = 0.7869$, $p > 0.05$ for day-1; $F(3.20) = 0.2586$, $p > 0.05$ for day-8; $F(3.20) = 0.5718$, $p > 0.05$ for day-15; $F(3.20) = 1.523$, $p > 0.05$ for day-22; $F(3.20) = 1.788$, $p > 0.05$ for day-29] followed by the Tukey-Kramer multiple-comparisons test. There were significant differences between the groups for bodyweight.

groups revealed that LPS treatment increased the TL significantly ($p < 0.001$) as compared with that in the control group, which explained the memory impairment resulting from LPS treatment. Intriguingly, compared with the LPS group, 30 days of pretreatment with ciproxifan (1 and 3 mg/kg) shortened the TL significantly ($p < 0.001$). Furthermore, the duration of TL reversal was comparable with that in the control group.

Ciproxifan Improved the Cognitive Functions of LPS-Treated Mice in the NOR Test

The effect of ciproxifan on the time spent exploring the novel object and discrimination abilities of LPS-treated mice in the NOR test is shown in Figure 5. In the TP, using two different objects (FO1 and NO), significant differences were observed between all groups for the time spent exploring the novel object [$F(3.20) = 15.82$, $p < 0.001$]. Comparison between the control group and the LPS group revealed a significant reduction ($p < 0.001$) of time spent exploring the novel object. However, significant ($p < 0.001$) improvements in the time spent exploring the novel object were noted after ciproxifan (1 and 3 mg/kg) treatment (Figure 5A).

Comparison among the groups revealed that treatment of ciproxifan and LPS altered the DI significantly [$F(3.20) = 26.97$, $p < 0.001$, one-way ANOVA] (Figure 5B). Mice administered LPS showed a significant ($p < 0.001$) reduction in the DI as compared with that in the control group. Pretreatment with ciproxifan (1 and 3 mg/kg) improved the DI significantly ($p < 0.001$) upon LPS treatment.

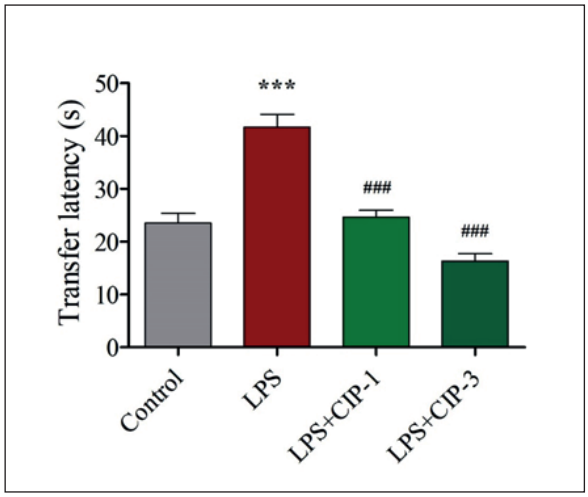

Figure 4. Effect of ciproxifan (CIP) on the transfer latency of lipopolysaccharide (LPS)-treated mice using the elevated plus-maze test. Results are the mean ± SEM (n = 6). One-way ANOVA [$F(3.20) = 35.32$, $p < 0.001$] followed by the Tukey-Kramer multiple-comparisons test. ***$p < 0.001$ compared with the control group; $^{###}p < 0.001$ compared with the LPS-treated group.

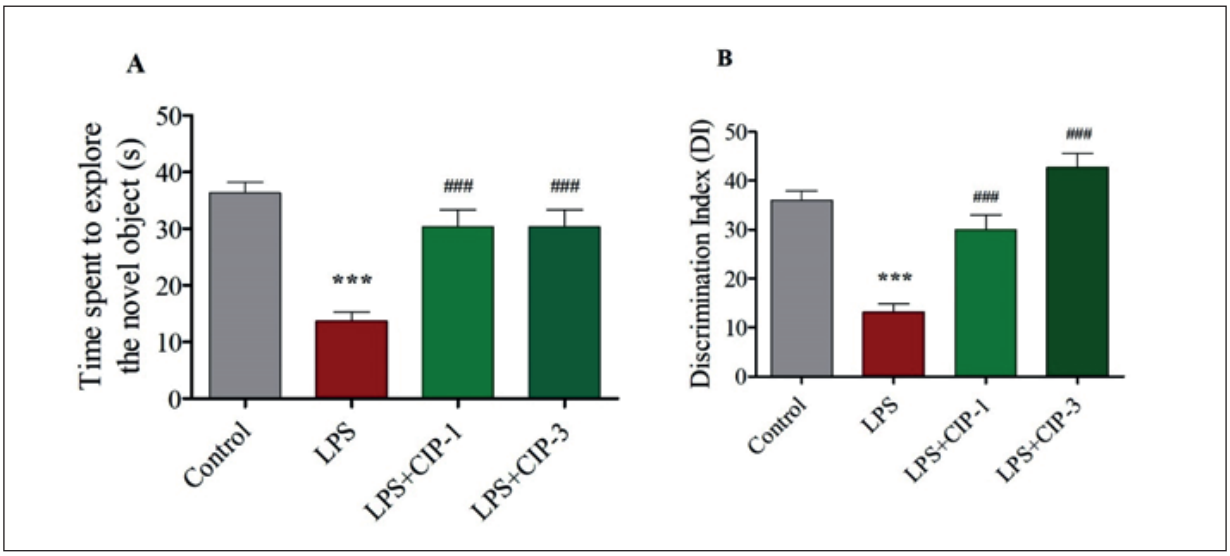

Figure 5. Effect of ciproxifan (CIP) on lipopolysaccharide (LPS)-induced cognitive impairment in mice using the novel object recognition test. **A**, Time spent exploring the novel object. **B**, Discrimination Index. Results are the mean ± SEM (n = 6). One-way ANOVA [$F(3.20) = 15.82$, $p < 0.001$ for the time spent exploring the novel object; $F(3.20) = 26.97$, $p < 0.001$ for the Discrimination Index] followed by the Tukey–Kramer multiple-comparisons test. ***$p < 0.001$ compared with the control group; $^{###}p < 0.001$ compared with the LPS-treated group.

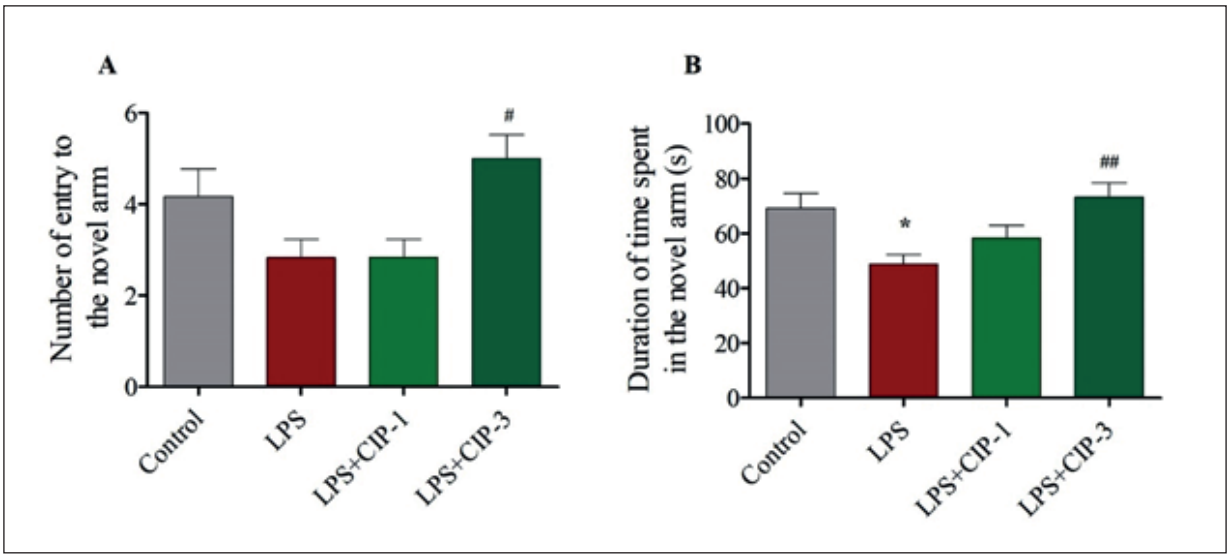

Figure 6. Effect of ciproxifan (CIP) on lipopolysaccharide (LPS)-induced cognitive impairment in mice using the Y-maze test. **A**, Number of entries to the novel arm. **B**, Duration of time spent in the novel arm. Results are the mean ± SEM (n = 6). One-way ANOVA [$F(3.20) = 4.789$, $p < 0.05$ for the number of entries to the novel arm; $F(3.20) = 5.353$, $p < 0.01$ for the duration of time spent in the novel arm] followed by the Tukey–Kramer multiple-comparisons test. *$p < 0.05$ compared with the control group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with the LPS-treated group.

Ciproxifan Improved the Cognitive Functions of LPS-Treated Mice in the Y-Maze Test

Figure 6 depicts the effect of ciproxifan on the behavioral parameters of mice with LPS-induced cognitive impairment in the Y-maze test. Significant differences were noted among groups for the number of entries into the novel arm [$F(3.20) = 4.789$, $p < 0.05$, one-way ANOVA]. LPS treatment resulted in a reduction in the number of entries into the novel arm, but not significantly so. A higher dose of ciproxifan (3 mg/kg) led to a comparable increase in the number of entries into the novel arm ($p < 0.05$) compared with that in the LPS-treated group (Figure 6A).

Figure 6B shows the duration of time spent in the novel arm during the T2 session of the Y-maze test. Comparison among groups showed significant differences [$F(3.20) = 5.353$, $p < 0.01$] in the duration of time spent in the novel arm. LPS-treated mice spent significantly ($p < 0.001$) less time in the novel arm compared with that by mice in the control group. Ciproxifan administration in LPS-treated mice led to a longer total time ($p < 0.001$) spent in the novel arm.

Ciproxifan Increased ACh Levels by Reducing AChE Activities in the Brains of LPS-Treated Mice

Figure 7 displays the effect of 30 days of ciproxifan administration on brain levels of ACh and AChE in different groups of LPS-treated mice. Using ELISA assay, there were significant differences in brain ACh levels among groups [$F(3.20) = 33.87$, $p < 0.001$] (Figure 7A, B). The ACh levels of LPS-treated mice were considerably lower ($p < 0.001$) than those of control mice. Pretreatment with ciproxifan (1 and 3 mg/kg) increased ACh levels in LPS-treated mice brains significantly ($p < 0.001$).

Changes in AChE levels [$F(3.20) = 20.18$, $p < 0.001$] were identified in the brain homogenates of mice in different groups. Compared with the control group, LPS treatment resulted in a significantly greater ($p < 0.001$) increase in AChE activity in brain brains. However, pretreatment with ciproxifan (1 and 3 mg/kg) reduced AChE activity in LPS-treated mice brains significantly ($p < 0.001$).

Discussion

Inflammation can lead to several complications, including cognitive dysfunction[17]. Several lines of evidence have revealed that LPS induces the release of proinflammatory cytokines to result in inflammation which, ultimately, induces cognitive impairment[18]. Furthermore, there is evidence of a link between prolonged inflammation and ACh levels in the brain[19]. A decrease in ACh levels in the brain has been reported to cause cognitive dysfunction[20].

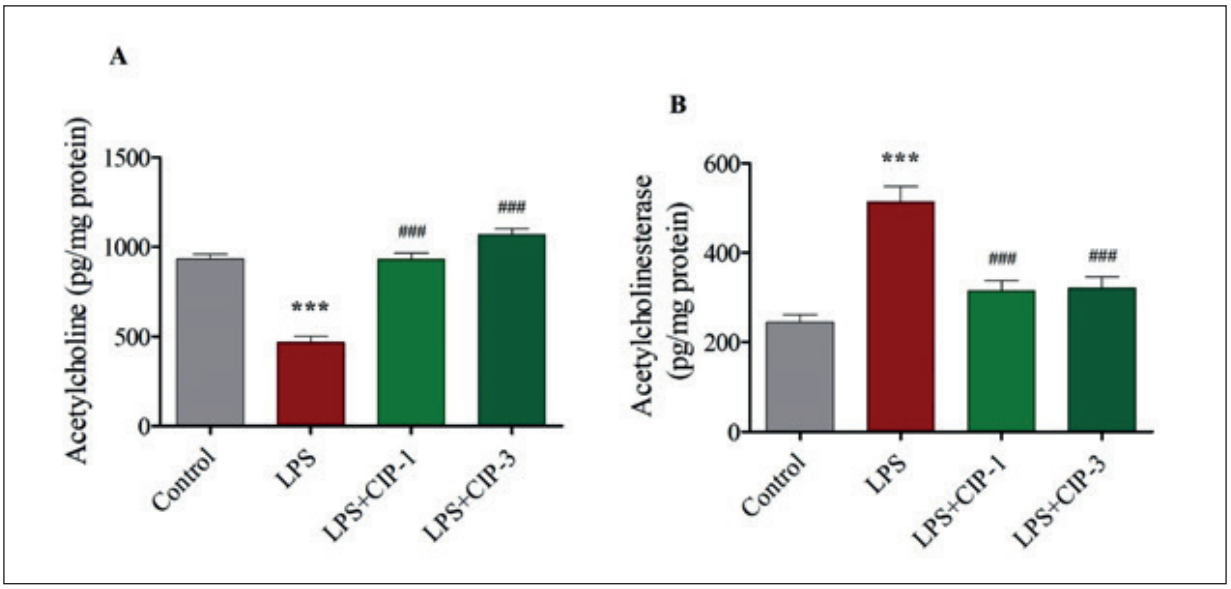

Figure 7. Effect of ciproxifan (CIP) on lipopolysaccharide (LPS)-induced cholinergic deficits in the mouse brain. **A**,Acetylcholine. **B**, Acetylcholinesterase. Results are the mean ± SEM (n = 6). One-way ANOVA [$F(3.20) = 33.87$, $p < 0.001$ for acetylcholine; $F(3.20) = 20.18$, $p<0.001$ for acetycholinesterase] followed by the Tukey–Kramer multiple-comparisons test. ***$p < 0.001$ compared with the control group; $^{###}p < 0.001$ compared with the LPS-treated group.

Ciproxifan is an antagonist of H3Rs (inhibitory receptors in the brain). Antagonism of H3Rs alters the levels of neurotransmitters, including ACh[21,22]. We investigated the neuroprotective effect of ciproxifan against inflammation-induced cognitive dysfunction in mice. We also evaluated the behavioral (Y-maze test, NOR test, and EPM test) and neurochemical (ACh and AChE levels) effects of ciproxifan in a mouse model of inflammation.

Using the Y-maze test, NOR test, and EPM test, we demonstrated memory impairment in mice treated with LPS, data which are consistent with results from other studies[23]. The Y-maze test showed a reduction in the number of entries and dwelling time in the novel arm, indicating that memory was impaired in LPS-treated mice compared with that in mice of the control group (Figure 6). Ciproxifan treatment reversed this memory impairment significantly. In addition, the exploration time spent on the novel object and the DI in the NOR test were reduced significantly in the LPS-treated group, whereas this reduction was reversed back to levels seen in the control group following ciproxifan treatment. Therefore, ciproxifan could be a therapeutic agent for the memory impairment caused by inflammation. According to the EPM test, the TL in the LPS-treated group was higher than that in the control group, which provided further proof of the cognitive impairment caused by inflammation. The TL recovered back to that observed in the control group following ciproxifan treatment. Taken together, these data indicated that memory was impaired in mice with inflammation induced by LPS, and that this impairment recovered back to normal levels after ciproxifan treatment.

ACh is an endogenous neurotransmitter present in central and peripheral nervous systems, and is involved in many physiological functions[24]. It exerts physiological activity by binding to two types of receptors[25]: nicotinic (ionotropic) and muscarinic (G protein-coupled)[26]. ACh and its receptors have essential roles in the regulation of cognitive function[24]. When ACh is released, it binds to nicotinic receptors in the hippocampus to promote its function and results in memory formation[27]. A reduction in ACh levels in the hippocampus is associated with age-related cognitive decline and some diseases (e.g., AD)[28]. Moreover, ACh release has been reported to be increased in the hippocampus during spatial-memory tasks[29]. In addition, a decrease in ACh levels in the hippocampus from the medial septum resulting from damage results in cognitive impairment[30]. We revealed that ACh levels were decreased in the brain of mice treated with LPS. Consistently, the activity of the hydrolyzing enzyme of ACh, AChE, was increased in LPS-treated mice. Preclinical studies[12,13] from our research team have demonstrated that LPS treatment alters the levels of ACh and AChE in animal brains. Interestingly, the levels of ACh and

AChE were brought back to normal levels following ciproxifan treatment in mice in the present study. Previously, we showed that ciproxifan treatment resulted in an increase in ACh levels in the brains of transgenic mice with AD (B6.129-Tg (APPSw)40BTLA/J mice)[3].

The current findings support ciproxifan's efficacy in reversing LPS-induced cognitive deficits by enhancing cholinergic transmission through antagonizing pre-synaptic H3R in cholinergic neurons. The conclusions reported from the current study are based on preliminary evaluations and will help in directing the researchers to explore additional benefits of ciproxifan on the management of memory deficits. Our study mainly focuses on the effect of drug on cholinergic neurotransmission. However, there are several other mechanisms involved in neuronal deficits including neuronal inflammation, oxidative vulnerability, deficits of other neurotransmitters, mitochondrial dysfunctions, and cellular apoptosis. Therefore, it will be interesting to explore other mechanisms which will provide additional support for the potential role of ciproxifan in memory dysfunctions. Furthermore, the study can be extended to the effect of ciproxifan on the specific areas of the brain such as the hippocampus, cortex, etc.

Conclusions

We demonstrated the beneficial effects of ciproxifan in mice suffering from cognitive impairment due to the inflammation induced by LPS. Enhancement of behavioral parameters (e.g., shortening of the TL in the EPM test; increased time spent exploring a novel object and a DI increase in the NOR test; increased number of entries to the novel arm and duration of time spent in the novel arm in the Y-maze test) were observed. Cholinergic activity was improved (increased ACh levels and decreased AChE activities in the brains of LPS-treated mice) upon ciproxifan treatment.

Our findings support the notion of ciproxifan reversing LPS-induced cognitive deficits in maze tests (EPM, NOR, and Y-maze) by enhancing cholinergic transmission thanks to antagonization of pre-synaptic H3Rs in cholinergic neurons. This conclusion is based on preliminary studies in mice, but our results may direct researchers to explore the benefits of ciproxifan in management of memory deficits related to neuronal disorders.

Funding

The present research was supported by the Deanship of Scientific Research, Qassim University, Saudi Arabia, under the project number 5601-pharmacy-2019-2-2-I during the academic year 1440AH/2019AD.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors gratefully acknowledge Qassim University, represented by the Deanship of Scientific Research on the financial support for this research under the number (5601-pharmacy-2019-2-2-I) during the academic year 1440AH/2019AD.

References

1) Zetterberg H, Bendlin BB. Biomarkers for Alzheimer's disease-preparing for a new era of disease-modifying therapies. Mol Psychiatry 2021; 26: 296-308.
2) Barbarino, P, Lynch, C, Bliss, A. From plan to impact III: maintaining dementia as a priority in unprecedented times. London: Alzheimer's Disease International, 2020.
3) Mani V, Jaafar SM, Azahan NSM, Ramasamy K, Lim SM, Ming LC, Majeed ABA. Ciproxifan improves cholinergic transmission, attenuates neuroinflammation and oxidative stress but does not reduce amyloid level in transgenic mice. Life Sci 2017; 180: 23-35.
4) Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM. Alzheimer's disease: targeting the cholinergic system. Curr Neuropharmacol 2016; 14: 101-115.
5) Ghamari N, Zarei O, Arias-Montaño JA, Reiner D, Dastmalchi S, Stark H, Hamzeh-Mivehroud M. Histamine H_3 receptor antagonists/inverse agonists: Where do they go? Pharmacol Ther 2019; 200: 69-84.
6) Cannon KE, Chazot PL, Hann V, Shenton F, Hough LB, Rice FL. Immunohistochemical localization of histamine H_3 receptors in rodent skin, dorsal root ganglia, superior cervical ganglia, and spinal cord: potential antinociceptive targets. Pain 2007; 129: 76-92.
7) Passani MB, Lin JS, Hancock A, Crochet S, Blandina P. The histamine H_3 receptor as a novel therapeutic target for cognitive and sleep disorders. Trends Pharmacol Sci 2004; 25: 618-625.
8) Bardgett ME, Davis NN, Schultheis PJ, Griffith MS. Ciproxifan, an H3 receptor antagonist, alleviates hyperactivity and cognitive deficits in the APP Tg2576 mouse model of Alzheimer's disease. Neurobiol Learn Mem 2011; 95: 64-72.
9) Abdelmelek H. *Pistacia lentiscus* oil attenuates memory dysfunction and decreases levels of biomarkers of oxidative stress induced by lipopolysaccharide in rats. Brain Res Bull 2018; 140: 140-147.

10) Lee J, Chan SL, Mattson MP. Adverse effect of a presenilin-1 mutation in microglia results in enhanced nitric oxide and inflammatory cytokine responses to immune challenge in the brain. Neuromolecular Med 2002; 2: 29-45.
11) Willard B, Hauss-Wegrzyniak B, Wenk GL. Pathological and biochemical consequences of acute and chronic neuroinflammation within the basal forebrain cholinergic system of rats. Neuroscience 1999; 88: 193-200.
12) Mohd Azahan NS, Mani V, Ramasamy K, Lim SM, Johari James RM, Alsharidah M, Alhowail A, Abdul Majeed AB. Mahanimbine-induced neuroprotection via cholinergic system and attenuated amyloidogenesis as well as neuroinflammation in lipopolysaccharides-induced mice. Phcog Mag 2020; 16: 57-63.
13) Rahim NS, Lim SM, Mani V, Hazalin NAMN, Majeed ABA, Ramasamy K. Virgin coconut oil-induced neuroprotection in lipopolysaccharide-challenged rats is mediated, in part, through cholinergic, anti-oxidative and anti-inflammatory pathways. J Diet Suppl 2020: 1-27.
14) Alharbi I, Alharbi H, Almogbel Y, Alalwan A, Alhowail A. Effect of metformin on doxorubicin-induced memory dysfunction. Brain sciences 2020; 10: 152.
15) Malik J, Kumar M, Deshmukh R, Kumar P. Ameliorating effect of lyophilized extract of *Butea frondosa* leaves on scopolamine-induced amnesia in rats. Pharm Biol 2013; 51: 233-239.
16) Tripathi A, Paliwal P, Krishnamurthy S. Piracetam attenuates LPS-induced neuroinflammation and cognitive impairment in rats. Cell Mol Neurobiol 2017; 37: 1373-1386.
17) Simen AA, Bordner KA, Martin MP, Moy LA, Barry LC. Cognitive dysfunction with aging and the role of inflammation. Ther Adv Chronic Dis 2011; 2: 175-195.
18) Wang WY, Tan MS, Yu JT, Tan L. Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. Ann Transl Med 2015; 3: 136.
19) Hampel H, Mesulam MM, Cuello AC, Farlow MR, Giacobini E, Grossberg GT, Khachaturian AS, Vergallo A, Cavedo E, Snyder PJ, Khachaturian ZS. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. Brain 2018; 141: 1917-1933.
20) Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM. Alzheimer's disease: Targeting the Cholinergic System. Curr. Neuropharmacol. 2016; 14: 101-115.
21) Hagenow S, Stasiak A, Ramsay RR, Stark H. Ciproxifan, a histamine H3 receptor antagonist, reversibly inhibits monoamine oxidase A and B. Sci Rep 2017; 7: 40541.
22) Bardgett ME, Davis NN, Schultheis PJ, Griffith MS. Ciproxifan, an H3 receptor antagonist, alleviates hyperactivity and cognitive deficits in the APP Tg2576 mouse model of Alzheimer's disease. Neurobiol Learn Mem 2011; 95: 64-72.
23) Swiergiel AH, Dunn AJ. Effects of interleukin-1beta and lipopolysaccharide on behavior of mice in the elevated plus-maze and open field tests. Pharmacol Biochem Behav 2007; 86: 651-659.
24) Picciotto MR, Higley MJ, Mineur YS. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. Neuron 2012; 76: 116-129.
25) Cisterna BA, Vargas AA, Puebla C, Fernández P, Escamilla R, Lagos CF, Matus MF, Vilos C, Cea LA, Barnafi E, Gaete H, Escobar DF, Cardozo CP, Sáez JC. Active acetylcholine receptors prevent the atrophy of skeletal muscles and favor reinnervation. Nat Commun 2020; 11: 1073.
26) Jones CK, Byun N, Bubser M. Muscarinic and nicotinic acetylcholine receptor agonists and allosteric modulators for the treatment of schizophrenia. Neuropsychopharmacology 2012; 37: 16-42.
27) Hasselmo ME. The role of acetylcholine in learning and memory. Curr Opin Neurobiol 2006; 16: 710-715.
28) Baxter MG, Frick KM, Price DL, Breckler SJ, Markowska AL, Gorman LK. Presynaptic markers of cholinergic function in the rat brain: relationship with age and cognitive status. Neuroscience 1999; 89: 771-779.
29) Stancampiano R, Cocco S, Cugusi C, Sarais L, Fadda F. Serotonin and acetylcholine release response in the rat hippocampus during a spatial memory task. Neuroscience 1999; 89: 1135-1143.
30) Herzog CD, Gandhi C, Bhattacharya P, Walsh TJ. Effects of intraseptal zolpidem and chlordiazepoxide on spatial working memory and high-affinity choline uptake in the hippocampus. Neurobiol Learn Mem 2000; 73: 168-179.