Effect of Chloroquine on Hyoscine-Induced Memory Impairment in Mice: Possible Involvement of Opioids and Nitric Oxide

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Abstract- Supporting evidence suggests the possible neuroprotective potential of chloroquine, an anti-malaria medication. Moreover, reports indicate that endogenous opioids and nitric oxide (NO) play role of a mediator by chloroquine's effects. In the present study, the effects of chloroquine on hyoscine-induced memory impairment were assessed. Furthermore, the possible involvements of opioids and NO were evaluated. Chloroquine was administered intraperitoneally (i.p.) at doses of 0.1, 0.5, 1, 3, 10, and 20 mg/kg to hyoscine-treated (1 mg/kg, i.p.) mice, and the spatial and fear memories were evaluated using Y-maze and passive-avoidance tasks, respectively. Also, to provide further evidence about chloroquine's mechanism of action, the opioid receptors and the NO production were blocked using two nonselective antagonist's naltrexone and L-NAME, respectively. Chloroquine at doses of 0.5, 10, and 20 mg/kg furtherly damaged the impaired memory of hyoscine-treated mice and at doses of 10 and 20 mg/kg impaired the memory of saline-treated mice in the passive avoidance task. Additionally, chloroquine at doses of 0.5 and 1 mg/kg improved the spatial memory in hyoscine-treated mice in the Y-maze test. In addition, naltrexone (3 mg/kg) reversed the neuroprotective effect of chloroquine (1 mg/kg) in hyoscine-treated mice in the Y-maze task. It could be concluded that chloroquine at low doses may improve cognitive performance by involving the opioid receptors; as a result, blocking the opioid receptors may reverse chloroquine's neuroprotective effect. Notably, chloroquine at high doses did not improve the memory, and in combination with hyoscine, it caused even more damage to long-term memory.

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Keywords: Chloroquine; Hyoscine; Memory impairment; Opioid receptors; Nitric oxide; Learning

Introduction

Alzheimer's disease (AD), as a progressive neurodegenerative disease and dementia's most abundant form (1), accelerates the impairment of memory and cognition (2) by the deposition of amyloid plaque (3), neurotransmitter dysregulation (4), inflammation, and neural loss in specific parts of the brain (5). One of the most notable pathophysiological features of AD is dysfunction of the cholinergic system (6). Today, acetylcholinesterase inhibitors like rivastigmine or donepezil are drugs of choice for symptomatic management of AD (7,8). An issue of particular concern is the low success and side effects of this therapy (9). Thus, more attempts should be made to find a more effective drug. In this study, Hyoscine-treated mice were used as a simple model of AD.

Chloroquine and its derivates have long been the first-line drugs in the treatment of malaria disease (10). Due to its immunosuppressive and anti-inflammatory characteristics, it has also been commonly used to treat a variety of autoimmune disorders such as rheumatoid

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arthritis, systematic lupus erythematosus (11,12), and several viral infections (13). Chloroquine could play a role in autophagy inhibition (14). In recent clinical trials, its effects on the central nervous system (CNS) has been investigated. Several reports have revealed that chloroquine can modify the neurodegenerative process in different ways, including suppressing pro-inflammatory cytokines in the microglial cells (15) and inhibiting glutamate-induced oxidative stress and cell death in CNS by acting on sigma-1 receptors (16). Thus, drugs like chloroquine could be a potential treatment for AD (16).

It is well established that the cholinergic neurons are involved in the process of memory and learning (17). Disturbances in learning, memory, and cognitive function in patients with dementia are caused predominantly by a defect in the cholinergic system (18). Hyoscine, also known as scopolamine, a muscarinic antagonist, interferes with both working and reference memories by blockade of the central cholinergic receptors (19). Therefore, scopolamine-induced dementia in rodents is used extensively in experiments concerned with the function of the cholinergic system in memory impairment and the investigation of probable therapeutic agents for AD (20).

The endogenous opioid system functions using four classes of receptors that activate the G proteins; mu (μ), delta (δ), and kappa (κ) (21). The opioid system is introduced as one of the most complex neural systems in the body. They are widely involved in various functions, including cell proliferation, ionic homeostasis, epileptic seizures, immune function, analgesia, and memory. The opioid receptors and their ligands can have immunosuppressive, immunostimulant or dual effects (22). Chloroquine might affect the CNS by activating the opioid system. It can lead to body scratching in rat and increase the release of the endogenous opioid peptides via stimulation of μ-opiate receptors (23). Various studies indicate an association between opioid system and memory. From the role of opioid receptors and peptides effect on stress-induced memory impairment (24) to its role in AD (25). According to the links between the opioid system and chloroquine's function along with the role of opioid system in memory and AD, we aimed to assess if chloroquine's effects on memory are opioid-dependent. For this purpose, we blocked the opioid receptors using an opioid receptor antagonist. Naltrexone is a non-selective opioid receptor antagonist. However, some doses of naltrexone show enhancing effects on the memory of mice (26), meaning it could interfere with our results; therefore, to make sure that naltrexone has neither enhancing nor worsening effects on the memory of mice, we added a naltrexone control group. Accordingly, we evaluated the participation of the opioid system in chloroquine's effects on memory of mice.

Along with the opioid system, there is another endogenous system that is related to both memory and chloroquine. Nitric oxide (NO), a known neurotransmitter in the CNS and a diffusible free radical gas, is a messenger that modulates neural functions. Nitric oxide synthase (NOS) produces NO from L-arginine (27). NO plays different physiological and pathological roles. It functions as a vasodilator, neuromodulator, and neurotransmitter in the CNS (28). NO pathway is related to the long-term hippocampal potentiation and developmental neural plasticity (29). NO modulates many CNS disorders, such as Parkinson's disease, Huntington disease, and epilepsy (27). There are three types of NOS isoforms that produce NO from the amino-acid L-arginine; endothelial NOS (eNOS), inducible NOS (iNOS), and neural NOS (nNOS). Chloroquine stimulates iNOS expression and increases NO level (28,30). The NO pathway can be inhibited by L-arginine analogs such as L-NAME (31). In this study, L-NAME is applied to block the NO synthesis and to answer the question of whether chloroquine alters the memory of mice using the NO pathway. Several studies indicate variant effects of this inhibitor on different aspects of memory, but we are still uncertain about role of L-NAME in memory. Some believe that treatment with L-NAME would impair memory formation (32); however, others report that L-NAME is more neuroprotective rather than neurotoxic (33,34). By adding a control group (receiving only L-NAME) to the study, we ensured that L-NAME had no interference with the memory tests.

Furthermore, evidence exists that a practical interaction between the NO pathway and the opioidergic system exists, and these two systems may play roles when it comes to chloroquine's modulatory effect on pentylenetetrazol (PTZ)-induced seizure in mice (35).

Accordingly, the aim of this study was to assess the impact of chloroquine on memory impairment induced by hyoscine, using passive avoidance and the Y-maze tasks in mice. Further, we aimed to determine involvement of the opioid system and NO pathway in the probable effects of chloroquine (either neuroprotective or neurodegenerative) on memory.

Materials and Methods

Animals and housing

Male mice weighing 20-30 grams at age 6-8 weeks were chosen, with each group containing 10 animals. Each mouse was used only once during the study. The animals were kept under standard laboratory conditions in the standard cages made of polycarbonate, under semi-steady temperature (23±2° C) and humidity (50±5%), with a 12 h light/dark cycle, and had free access to food and water. The study was performed in accordance with the guidelines for the Care and Use of Laboratory Animal Ethics Committee of Tehran University of Medical Sciences (Ethics ID: IR.TUMS.MEDICINE.REC.1400.319) as well as the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

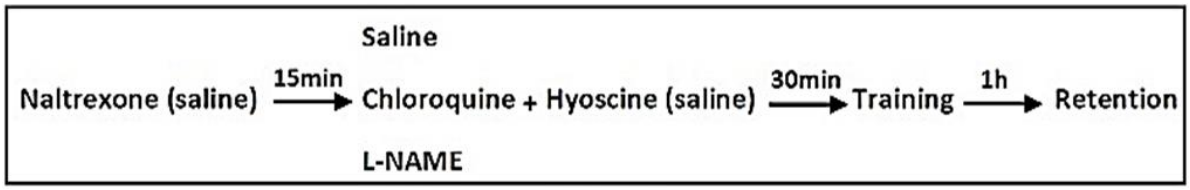
Drugs

Hyoscine (scopolamine), chloroquine, naltrexone and L-NAME were used in this study and purchased from Sigma-Aldrich (Germany). The drugs were injected intraperitoneally (i.p.) after dissolving in physiological saline solution.

Experimental design

Hyoscine (scopolamine) at a dose of 1 mg/kg was administered to mice to impair memory. Then, chloroquine was injected at various doses (0.1, 0.5, 1, 3, 10, 20 mg/kg). Naltrexone (3 mg/kg) was used for blockade of the opioid receptors, and L-NAME (10 mg/kg) was used to inhibit the nitric oxide synthases.

In the treatment groups, we induced memory impairment using hyoscine (1 mg/kg, i.p.). Hyoscine, chloroquine, and L-NAME were injected 30 minutes prior to the training trial in Y-maze (the following schema) and passive avoidance tests, and naltrexone was administered 15 minutes in advance (45 minutes before the training).



The control group received saline. Four treatment groups separately received hyoscine (1 mg/kg), naltrexone (3 mg/kg), and L-NAME (10 mg/kg) to make sure none of these substances altered the memory.

Chloroquine-treated groups were used to test whether or not the effective chloroquine doses have any impact on the non-impaired memory of mice. Further addition of naltrexone and L-NAME to the experimental groups was done only for those doses of chloroquine, which had shown either enhancing or worsening effects on impaired memory.

Behavioral assessments

Y-maze task

Y-maze, as a behavioral test, aims to evaluate rodents' spatial learning and memory based on the fact that rodents tend to explore the new arm instead of revisiting previously explored arms (36,37). The Y-maze was made of three identical arms at a 120° angle from each other, each showing exclusive visual clues. Each mouse was placed in the start arm for the first time, with one arm closed (novel arm), and was given 10 minutes to freely explore two open arms (training). After an hour interval, the same mouse was placed in the start arm again allowed to explore all of the three arms (start, novel, other) for 8 minutes (retention). During the retention trial, exploration time in each arm was recorded, as well as the percentage of the number of entries into each arm. Total number of entries into the arms would indicate the locomotor activity of mice. Recognizing the novel arm is considered as improving spatial recognition memory.

Passive avoidance task

Previously explained passive avoidance task (37,38) was another behavioral test performed in this study to assess long-term memory and learning. The passive avoidance apparatus contains a two-compartment (light and dark) box (20×20×20 cm), separated by a guillotine door (Borj Sanat Company, Tehran, Iran). The mouse was placed in the white compartment with the guillotine door closed for 10 seconds to get familiar with the box. After opening the door, the mouse was given a maximum of 300 seconds to step through the dark compartment, crossing the guillotine door. When completely crossed, the mouse was given an electric shock from the foot (0.5 mA, 1 s) and then taken out of the apparatus (acquisition/training). After 24 h interval,

the same mouse was placed in the same white compartment with the guillotine door open (the retention). Mice with better memory spend more latency time entering the dark compartment and avoid getting into it for the second time. In both acquisition and retention trials, the latency time was recorded, and the cut-off time was set to 300 seconds.

Statistical analysis

The variables which are used in statistical analysis are as follows: 1) Duration of the arm visits (sec.) expresses the mean time spent by mice exploring in each arm during 8-min trial, 2) number percentage of the arm visits in an 8-min trial, and 3,) number of the arm visits is the total number of entries that expressing the locomotor activity of mice, are the results reported in the Y-maze test. 4). The latency time (sec.), is the time that takes the mice to enter the dark compartment of the passive avoidance apparatus, measured during both acquisition and retention trials.

Data was analyzed using SPSS statistical software package (version 25) and described as mean±SEM. $P<0.05$ was considered statistically significant. Duration of arm visits (sec.) and percentage of number of arm entries were compared between experimental groups using multivariate analysis of variance (ANOVA) and Tukey post-test. One-way ANOVA analysis was used for comparison of locomotor activity and within-groups' differences in exploration time and arm entries for each special treatment. Data from passive avoidance test, latency time (s) were analyzed by the non-parametric ANOVA using a medians test. We also used Paired-Samples T Test for within-group comparison of acquisition trial with retention trial in each group.

Results

The Y-maze test

Effects of chloroquine and hyoscine on spatial memory of normal mice in the Y-maze test

Chloroquine was given at different doses (0.5, 1, and 3 mg/kg) (Figure 1). Within-group comparisons with the novel arm showed that the saline-treated group spent significantly lower time in the other arm ($P<0.001$) (Figure 1A). Besides, the number percentages of the other arm entries were markedly lower ($P<0.001$) (Figure 1B). Chloroquine group at a dose of 3 mg/kg spent considerably less time in the corresponding other arms ($P<0.001$), and at a dose of 0.5 mg/kg, the mice spent significantly less time in the start arm ($P<0.01$) (Figure 1A). However, between-group comparisons demonstrated no significant differences compared to the novel arms of chloroquine-treated groups with the novel arm of the saline-treated group in cases of the duration and number percentages of arm visits (Figures 1A and B). Moreover, the total number of arm visits was remarkably augmented by chloroquine at doses of 1 and 3 mg/kg, $P<0.001$ and $P<0.05$, respectively (Figure 1C).

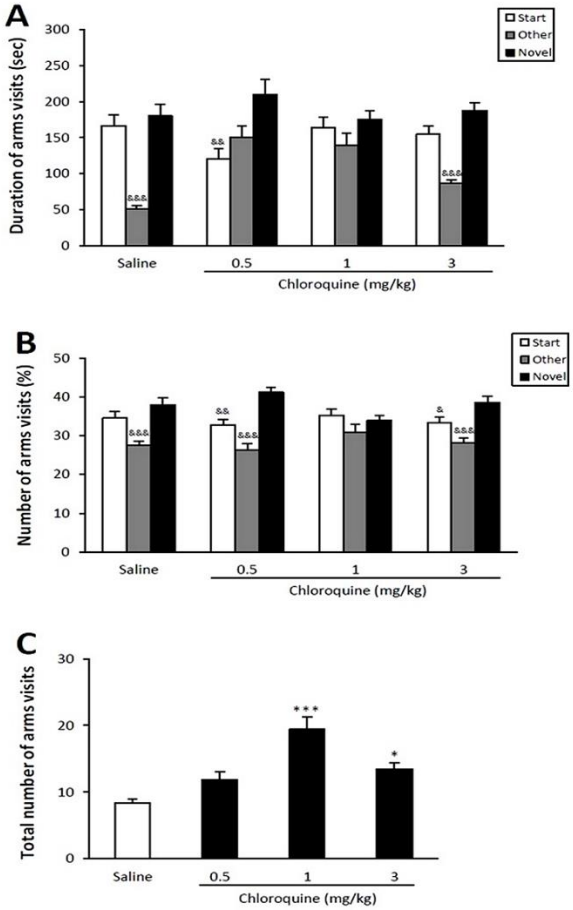


Figure 1. Effect of chloroquine on the acquisition of spatial recognition memory and motor activity in the Y-maze task. Chloroquine (0.5, 1, 3 mg/kg, i.p.) or saline was injected 30 min before the training session into four groups. 10 different mice were tested in the treatment groups. Exploration time of mice through each arm (A), the percentage entries into each arm (B), and the total number of arm entries (c) were recorded during the 8-min test. Data are represented as the Mean ±SEM. (&&$P<0.01$ and &&&$P<0.001$ within-group comparison with the novel arm; *$P<0.05$ and ***$P<0.001$ compared with saline group)

Effect of chloroquine on hyoscine-induced impairment of spatial memory acquisition in the Y-maze test

Effects of different doses of chloroquine (0.1, 0.5, 1, 3, 10 and 20 mg/kg, i.p.) on hyoscine-induced memory impairment (1 mg/kg, i.p.) are shown in figure 2.

Within-group comparisons with the novel arm showed that the hyoscine group (1 mg/kg) spent considerably more time in the start arm (P<0.001) (Figure 2A). However, between-group comparisons demonstrated that the hyoscine-treated group spent significantly less time in the novel arm (P<0.05) compared to the saline-treated group (Figure 2A). Moreover, the hyoscine (1 mg/kg) group had significantly higher number of start arm entries (P<0.05) (Figure 2B). Likewise, the total number of arm visits was remarkably augmented by hyoscine P<0.001 (Figure 2C).

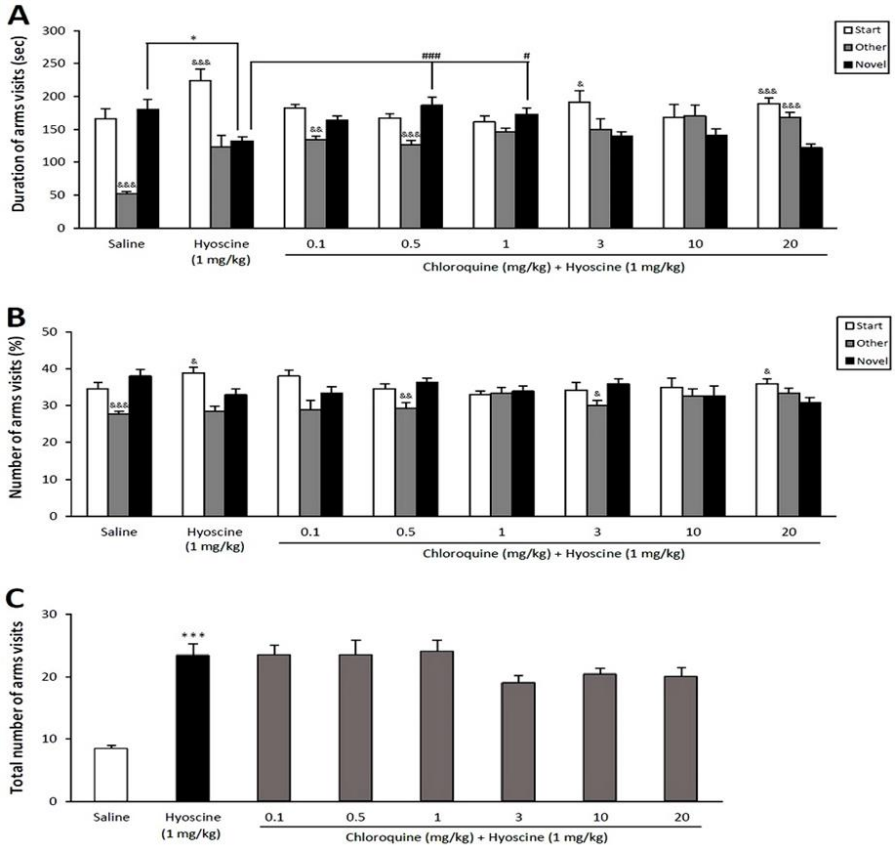


Figure 2. Effect of chloroquine on hyoscine-induced impairment of spatial memory acquisition. Chloroquine (0.1, 0.5, 1, 3, 10, 20 mg/kg, i.p.) and hyoscine (1 mg/kg, i.p.) were injected 30 min prior to the training session in seven groups. Ten different mice were tested each time in treatment groups. Exploration time of mice through each arm (A), the percentage of entries to each arm (B), and the total number of entries to arms (c) were recorded during the 8-min test. Data are represented as the mean ±SEM. (&P<0.05, &&P<0.01 and &&&P<0.001 within-group comparison with the novel arm; *P<0.05 and ***P<0.001 compared with saline group; #P<0.05 and ###P<0.001 compared with hyoscine-treated group)

Within-group comparisons with the corresponding novel arms demonstrated that chloroquine-treated groups at doses of 0.1 and 0.5 mg/kg spent markedly less time in the other arms, P<0.01 and P<0.001, respectively. In addition, chloroquine-treated groups at a dose of 3 mg/kg spent considerably more time in the start arm (P<0.05), and at a dose of 20 mg/kg, the mice spent significantly more time in the start and other arms (P<0.001), (Figure 2A). Between-group comparisons with the hyoscine-treated group demonstrated that chloroquine-treated groups at doses of 0.5 and 1 mg/kg spent considerably more time in the corresponding novel arms P<0.001 and P<0.01, respectively (Figure 2A). Within-group comparisons with the corresponding novel arms demonstrated that chloroquine-treated groups at doses of 0.5 and 3 mg/kg had considerably lower numbers percentage of the other arms, P<0.01 and P<0.05, respectively. Further, the chloroquine-treated group at a dose of 20 mg/kg showed a markedly higher percentage of the number of the start arm entries (P<0.05) (Figure 2 B).

Effects of naltrexone and L-NAME on the acquisition of spatial recognition memory in the Y-maze test

Naltrexone (3 mg/kg) and L-NAME (10 mg/kg) were given to normal mice. Within-group comparisons

with the corresponding novel arms demonstrated that L-NAME (P<0.01) and naltrexone (P<0.01) groups spent significantly less time in the other arms (Figure 3A). Moreover, a significantly lower number of other arm entries were observed in L-NAME (P<0.01) groups (Figure 3B).

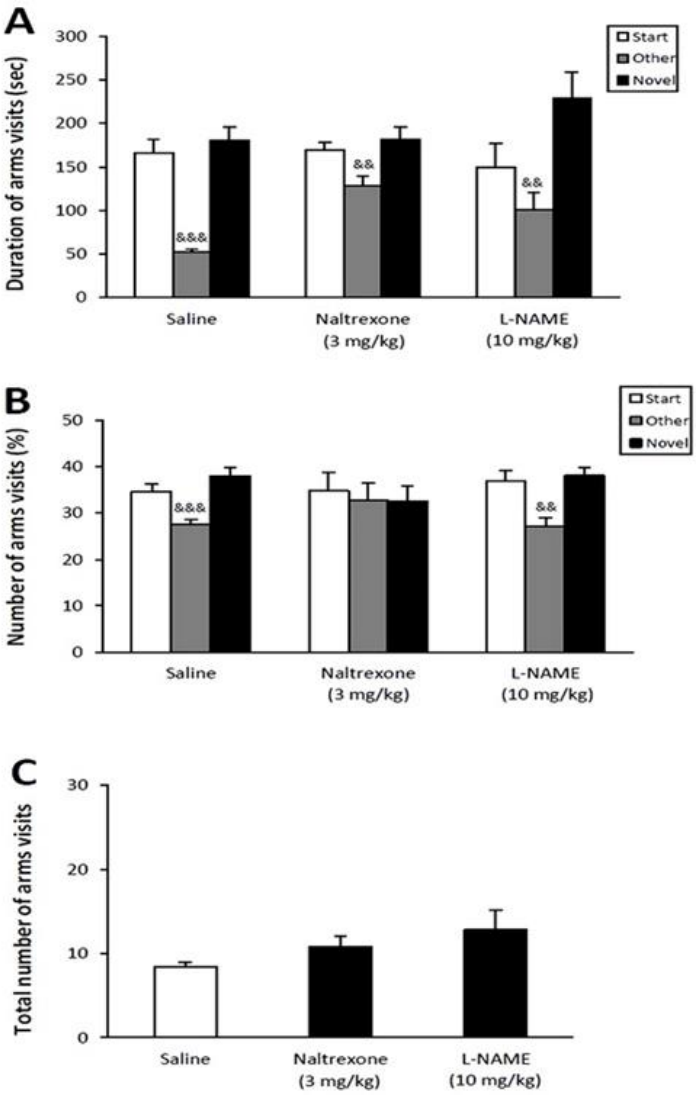


Figure 3. Effects of naltrexone and L-NAME on the acquisition of spatial recognition memory. L-NAME (10 mg/kg, i.p.) and naltrexone (3 mg/kg, i.p.) was administered to separate groups, 30 min and 45 min prior to the training session, respectively. Ten different mice were tested each time in the treatment groups. Exploration time of mice through each arm (A), the percentage of entries to each arm (B), and the total number of entries to arms (c) were recorded during the 8-min test. Data are represented as the mean ± SEM (&&P<0.01 and &&&P<0.001 within-group comparisons with the novel arm)

Effect of naltrexone and L-NAME on chloroquine-treated mice in the Y-maze task

Chloroquine at a dose of 0.5 mg/kg was given to hyoscine-treated mice. They also received naltrexone (3 mg/kg) and L-NAME (10 mg/kg). Within-group comparisons with the corresponding novel arms demonstrated that the chloroquine (0.5 mg/kg) -the treated group spent markedly lower time in the other arm (P<0.001). Further, the chloroquine (0.5 mg/kg)+naltrexone (3 mg/kg) group spent markedly lower time in the other and start arms, P<0.001 and P<0.05, respectively (Figure 4A). Between-group comparisons demonstrated that the hyoscine-treated group, which received chloroquine (0.5 mg/kg), spent more time in the novel arm (P<0.001) compared to the hyoscine-treated group (Figure 4A). Regarding the percentages of the number of arm entries, within-group comparisons with the novel arms demonstrated that

memory-impaired groups which received chloroquine (0.5 mg/kg) or chloroquine (0.5 mg/kg)+naltrexone (3 mg/kg) had lower numbers of the other arm entries (P<0.01) (Figure 4B).

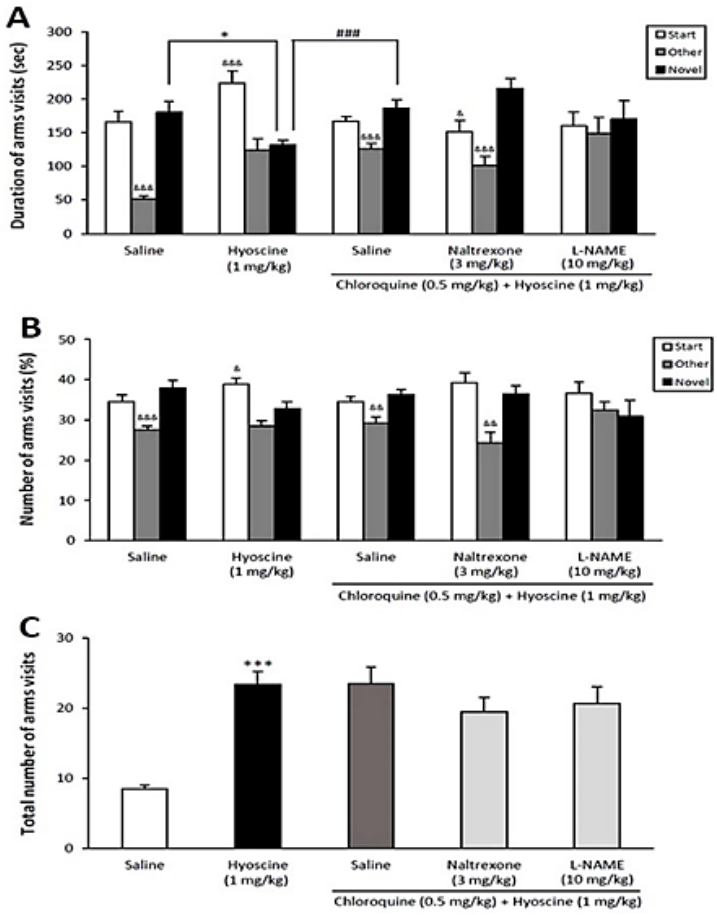


Figure 4. Influences of naltrexone and L-NAME on chloroquine (0.5 mg/kg) effects in the acquisition of spatial recognition memory. Chloroquine (0.5 mg/kg, i.p.) was injected 30 min before the training session, and memory impairment was induced using hyoscine (1 mg/kg, i.p.) 30 min before the training session. Naltrexone (3 mg/kg, i.p.) was injected 15 min before administration of chloroquine and hyoscine. In another group, L-NAME (10 mg/kg, i.p.) was injected simultaneously with chloroquine and hyoscine. Ten different mice were tested each time in the treatment groups. Exploration time of mice through each arm (A), the percentage of entries to each arm (B), and the total number of entries to arms (c) were recorded during the 8-min test. Data are represented as the mean±SEM. (&P<0.05, &&P<0.01 and &&&P<0.001 within-group comparison with the novel arm; *P<0.05 and ***P<0.001 compared with the saline group; ###P<0.001 compared with hyoscine-treated group)

Chloroquine at a dose of 1 mg/kg was also given to hyoscine-treated mice, which received naltrexone (3 mg/kg) and L-NAME (10 mg/kg) (Figure 5). Within-group comparisons to the novel arms demonstrated that the chloroquine (1 mg/kg)+naltrexone (3 mg/kg) group spent markedly more time in the other and start arms, P<0.01 and P<0.05, respectively (Figure 5A). Between-group comparisons with the hyoscine-treated group demonstrated that the hyoscine (1 mg/kg)+chloroquine (1 mg/kg) group spent more time in the novel arm (P<0.05). Furthermore, comparison with hyoscine (1 mg/kg)+chloroquine (1 mg/kg) group showed that chloroquine+naltrexone (3 mg/kg) group spent significantly less time in the novel arm (P<0.001) (Figure 5A). Additionally, the hyoscine-treated group which received chloroquine (1 mg/kg) and L-NAME (10 mg/kg) had lower numbers of the other arm entries (P<0.001) (Figure 5B).

The passive avoidance test

Effect of chloroquine and hyoscine on non-impaired memory in the passive avoidance task

Figure 6 illustrates the effects of chloroquine on step-through latency in the passive avoidance task. Chloroquine (0.5, 3, 10, 20 mg/kg, i.p.) was injected 30 min before the shock session (the first session of the

test) (Figure 6A). Chloroquine at doses of 10 and 20 mg/kg significantly reduced the retention time in comparison with the retention trial of the control group (saline-treated), $P<0.001$ and $P<0.05$, respectively. Additionally, a within-group comparison of the retention trial with the acquisition trial demonstrated that the latency time of retention trial raised significantly in saline-treated group ($P<0.001$). Similarly, the latency time of retention trial was enhanced markedly in chloroquine-treated groups at doses of 0.5, 3, and 20 mg/kg, $P<0.001$, $P<0.001$, and $P<0.01$, respectively (Figure 6A).

Notably, hyoscine significantly decreased the retention period in the passive avoidance task in comparison with the retention trial of the control group ($P<0.001$) (Figure 6B). In comparison to the retention trial of the hyoscine-treated group, chloroquine at doses of 0.5, 10, and 20 mg/kg had considerably lower latency time in the retention trials, $P<0.01$, $P<0.05$, and $P<0.001$, respectively. Furthermore, chloroquine at doses of 0.5, 1, 10, and 20 mg/kg markedly decreased the latency time in the retention trial in the hyoscine-treated group ($P<0.05$) (Figure 6B).

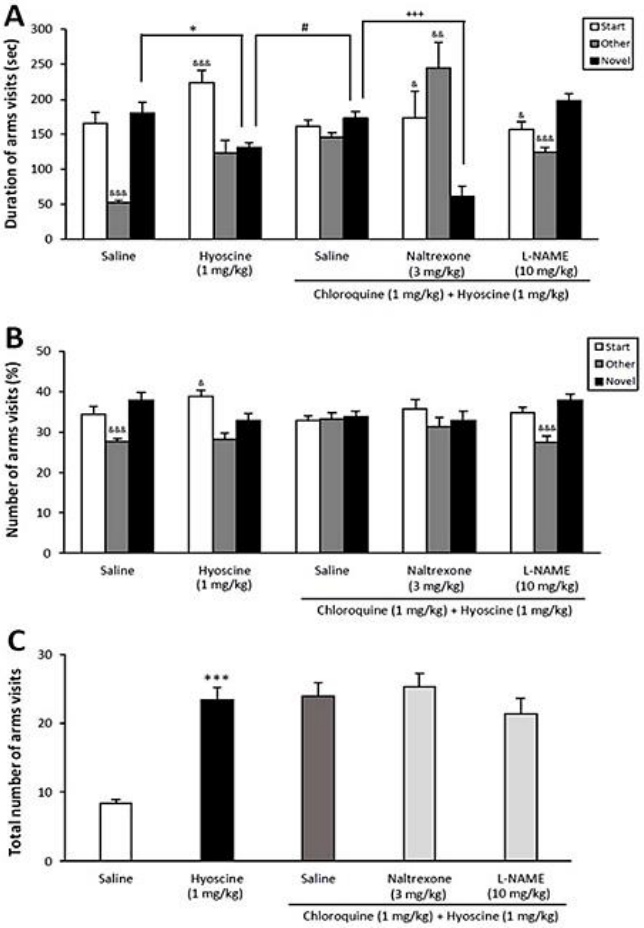


Figure 5. Influences of naltrexone and L-NAME on chloroquine (1 mg/kg) effects in the acquisition of spatial recognition memory. Chloroquine (1 mg/kg, i.p.) was injected 30 min prior to training session, and memory impairment was induced using hyoscine (1 mg/kg, i.p.) 30 min before the training session. Naltrexone (3 mg/kg, i.p.) was injected 15 min prior to chloroquine and hyoscine administration. In another group, L-NAME (10 mg/kg, i.p.) was injected at the same time with chloroquine and hyoscine. 10 different mice were tested each time in the treatment groups. Exploration time of mice through each arm (A), the percentage of entries to each arm (B), and the total number of entries to the arms (c) were recorded during the 8-min Test. Data are represented as the mean±SEM (&$P<0.05$, &&$P<0.01$ and &&&$P<0.001$ within-group comparison with the novel arm; *$P<0.05$ and ***$P<0.001$ compared with the saline group; #$P<0.05$ compared with hyoscine-treated group; +++$P<0.001$ compared with chloroquine-hyoscine-treated group)

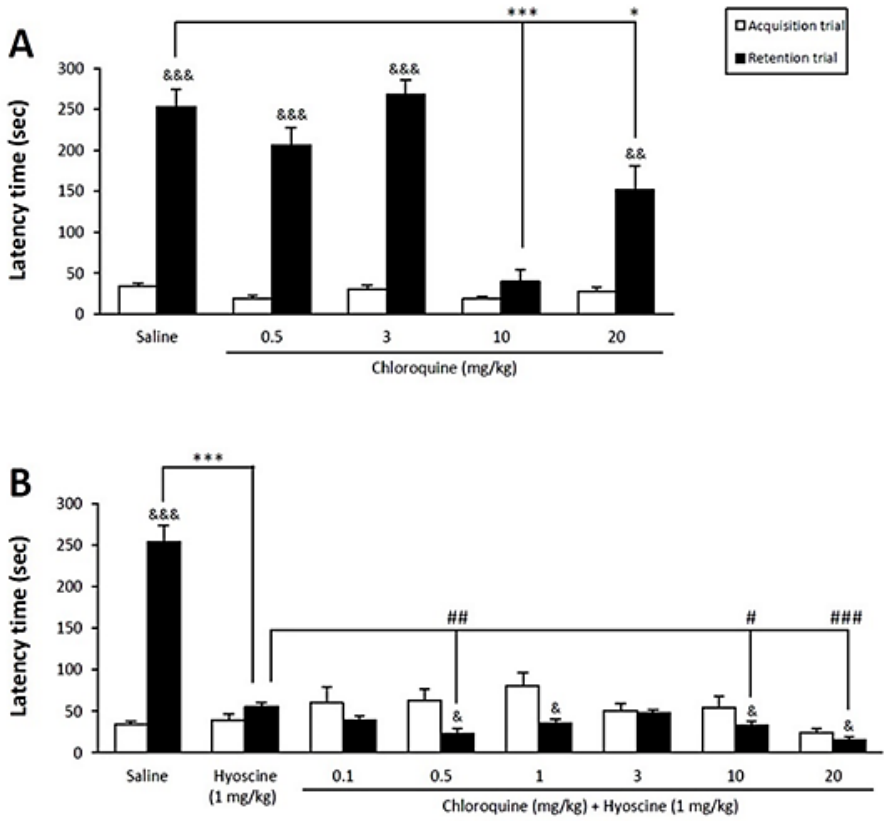


Figure 6. Effect of chloroquine on step-through latency in the passive avoidance task. Chloroquine (0.5, 3, 10, 20 mg/kg, i.p.) was injected 30 min before the shock session (A). Effects of chloroquine on hyoscine-induced step-through latency impairment in the passive avoidance task. Chloroquine (0.1, 0.5, 1, 3, 10, 20 mg/kg, i.p.) and hyoscine (1 mg/kg, i.p.) were injected 30 min prior to the shock session (B). Ten different mice were tested each time in the treatment groups. The latency time for the mice to enter the dark compartment was recorded. Data are represented as the mean±SEM (&*P*<0.05, &&*P*<0.01, &&&*P*<0.001 within-group comparison of the retention trial with the acquisition trial; **P*<0.05, ****P*<0.001 compared with the saline group; #*P*<0.05, ##*P*<0.01, ###*P*<0.001 compared with the hyoscine-treated group)

Effects of naltrexone and L-NAME on impaired memory in the passive avoidance task

Effects of naltrexone and L-NAME on chloroquine-treated mice (0.5, 10, and 20 mg/kg) in which the memory was impaired by hyoscine (1 mg/kg) are illustrated in figure 7. As it can be observed, the hyoscine-treated group had a significantly lower latency time of retention trial in comparison with the saline group (*P*<0.001) (Figure 7A). Remarkably, chloroquine at doses of 0.5, 10, and 20 mg/kg markedly reduced the latency time of retention trials in hyoscine-treated mice, *P*<0.01, *P*<0.05, and *P*<0.001, respectively, compared to the hyoscine-treated group (Figures. 7 A, B and C). Nevertheless, in comparison to the hyoscine-treated group, which also received chloroquine (0.5, 10, and 20 mg/kg), neither naltrexone (3 mg/kg) nor L-NAME (10 mg/kg) could influence chloroquine's (0.5, 10 and 20 mg/kg) effects on the fear-conditioned memory (Figure 7A, B and C). In addition, within-group comparisons showed that the saline group had significantly higher latency time in the retention trial (*P*<0.001) while chloroquine-treated groups had considerably lower latency time (*P*<0.05) (Figure 7A, B and C).

Naltrexone (3 mg/kg) and L-NAME (10 mg/kg) administration did not significantly influence the latency time in normal mice compared to the saline-treated group (Figure 7D). Moreover, a within-group comparison of the retention trial with the acquisition trial demonstrated that the latency time in the retention trials raised significantly in the saline-treated group (*P*<0.001) as well as naltrexone (3 mg/kg) and L-NAME (10 mg/kg)-treated groups (*P*<0.001) (Figure 7D).

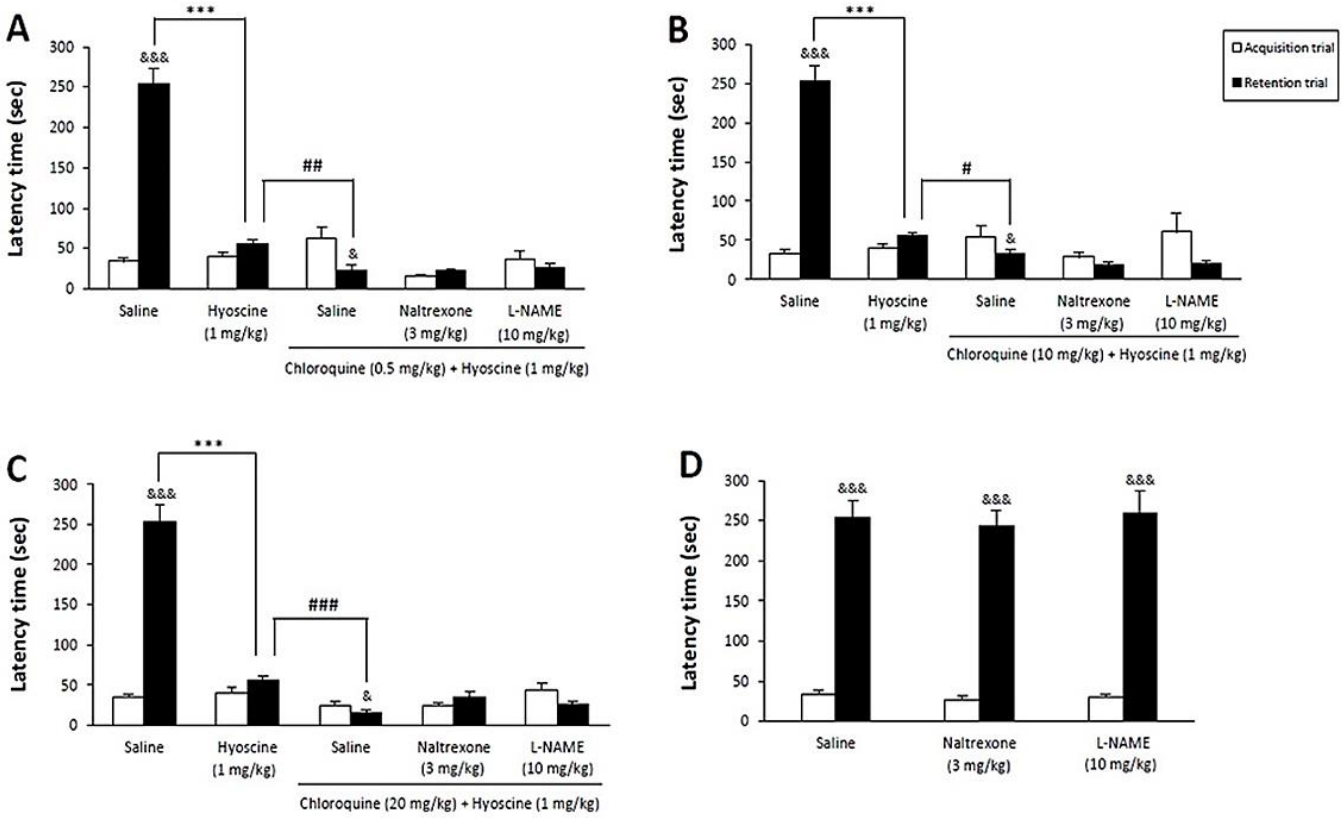


Figure 7. Influences of naltrexone and L-NAME on chloroquine effects on hyoscine-treated groups in the passive avoidance task. Chloroquine (0.5, 10, 20 mg/kg, i.p.) and hyoscine (1 mg/kg, i.p.) was injected 30 min before starting the first session of the test (A, B, and C). Naltrexone (3 mg/kg, i.p.) was injected 15 min prior to chloroquine and hyoscine administration. L-NAME (10 mg/kg, i.p.) was injected simultaneously with chloroquine and hyoscine. Effect of naltrexone and L-NAME on step-through latency in the passive avoidance task (D). Naltrexone (3 mg/kg, i.p.) was injected 45 min before the first session of the test. In another group, L-NAME (10 mg/kg, i.p.) was administered 30 min prior to the acquisition trial (first day) (D). 10 different mice were tested in the treatment groups. The latency time to enter the dark compartment was recorded. Data are represented as the mean±SEM (&*P*<0.05 and &&&*P*<0.001 within-group comparisons of the retention trial with the acquisition trial; ****P*<0.001 compared with the saline group; #*P*<0.05, ##*P*<0.01 and ###*P*<0.001 compared with the hyoscine-treated group)

Discussion

In the current experimental study, we evaluated the effects of chloroquine at various doses on the impaired memory of mice. Accordingly, the spatial and fear memories were assessed in the Y-maze and the shuttle boxes, respectively. Results of chloroquine administration on hyoscine-induced dementia of mice are contradictory; chloroquine showed both enhancing and worsening effects on the impaired memory of mice. We concluded that the effects of intraparietal injection of chloroquine in mice with hyoscine-induced amnesia follow a dose-dependent manner.

The effect of chloroquine on memory found in this study is not its only dose-dependent function. As an example of chloroquine's dose-dependent functions, earlier investigations have revealed that chloroquine dose-dependently reduces the secretion of proinflammatory cytokines, such as IL-6 (39).

In the present study, use of chloroquine at doses of 0.5 and 1 mg/kg improved the impaired memory in the Y-maze task. However, chloroquine at doses of 0.5, 10, and 20 mg/kg caused a deficit in the long-term memory, To determine whether the memory-improving characteristic of chloroquine was mediated through the opioid receptors, naltrexone, an antagonist of the opioid receptors, was applied. The ameliorative effect of chloroquine (1 mg/kg) was abolished with naltrexone, while naltrexone at a dose of 3 mg/kg had no noticeable effect on memory.

Among the doses of chloroquine in this trial, only the effect of chloroquine at a dose of 1 mg/kg showed significant involvement of the opioid receptors, given that administration of the opioid antagonist reversed the

neuroprotective effect of chloroquine at this dose. Conversely, administration of L-NAME had no significant influence on chloroquine's functions, indicating that NO pathway was involved in neither improving nor worsening effects of chloroquine on memory.

Chloroquine's enhancing effect on impaired memory of mice is just one of chloroquine's opioid-related effects. A recent study indicates that co-administration of naltrexone and chloroquine attenuates the chloroquine-induced phospholipidosis complications. They also demonstrated the probable involvement of the endogenous opioid system in this process (40).

Chloroquine-induced pruritus is one of the most common side effects of chloroquine and might decrease compliance of patients (41). In patients having this complication, naltrexone administration showed antipruritic effects. Therefore, it could be concluded that the opioid receptors, specifically μ receptors, are related to chloroquine-induced itch sensation (42). Accumulating evidence revealed that the opioid system is a mediator in itching caused by chloroquine (23).

Moreover, chloroquine-induced pruritus is associated with the NO pathway in the skin. Sumatriptan, a 5-hydroxytryptamine 1b/1d receptor agonist (5-HT1b/1d), suppresses chloroquine-induced itch (43). Chloroquine provokes scratching at least partly via the NO/cGMP pathway and the role of nNOS is more significant than iNOS and eNOS (44).

In a recent study, chloroquine has shown regulating effects on PTZ-induced seizure in mice. The seizure threshold was significantly increased by chloroquine (5 mg/kg). Additionally, the reported anticonvulsant effect of chloroquine was reversed using naltrexone (1 mg/kg), as this effect was fully hindered by acute simultaneous administration of L-NAME (5 mg/kg) or a selective neuronal NOS inhibitor 7-NI (40 mg/kg), along with the effective dose of chloroquine. Interestingly, when the lower doses of naltrexone (0.1 mg/kg) and 7-NI (15 mg/kg) were combined, a stronger blockade was seen, in terms of the chloroquine's anticonvulsant effect. The measured levels of nitrite in hippocampus were increased after administration of chloroquine at a dose of 5 mg/kg. Also, chloroquine has lowered the seizure threshold at a dose of 20 mg/kg, which was inhibited by appliance of L-NAME (5 mg/kg), 7-NI (40 mg/kg) and naltrexone (1 mg/kg). In conclusion, the NO signaling could play a mediatory or modulatory role in opioid-dependent anticonvulsant effects of chloroquine. It seems that the NO and the opioid systems are involved in the mechanisms through which chloroquine lowers the threshold in seizures induced by PTZ (35).

The interaction between the opioidergic and the nitrergic systems has been estimated in different conditions such as memory acquisition in cholestatic mice (45), the positive effect of acute chloroquine administration on PTZ-induced convulsion in mice (35), resistance depression in bile duct ligated (BDL) mice (46) and gastric damage induced by ethanol in cholestatic rats (47).

As explained above, some effects of chloroquine are known to be concerned with both the opioid system and the NO pathway. In this study, nevertheless, we failed to show any connection between chloroquine's effect on memory and the NO pathway. Current findings indicate that chloroquine may alter the impaired memory of mice via the opioid system. However, further investigations are needed to fully understand chloroquine's mechanism of action on memory.

Evaluation of chloroquine's effects on memory and learning in this study showed dose-dependent patterns. When assessed by Y-maze, the impaired memory of mice was enhanced by low doses of chloroquine (0.5 and 1 mg/kg). Among these doses, only the memory-enhancing influence of the higher dose (1 mg/kg) was dependent on the opioid receptors and was countered after the blockade of the opioid receptors by naltrexone. Chloroquine's worsening effects on memory showed a dose-dependent manner as well. In the passive avoidance task, three different doses of chloroquine (0.5, 10, and 20 mg/kg) furtherly damaged impaired memory of mice. Therefore, chloroquine may increase cognitive ability at low doses by involving the opioid receptors, and blocking the opioid receptor may play a role in reversing its effect. Nevertheless, chloroquine at high doses and in combination with hyoscine causes more damage to long-term memory.

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