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Comparative efficacy of haloperidol and placebo for treatment of delirium induced by biperiden and LPS in adult male rats based on their learning style and change in their short term spatial memory

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ABSTRACT

Background and Objectives: Delirium refers to acute loss of consciousness and is characterized by confused thinking and impaired orientation. It is a life-threatening, but reversible syndrome manifested by cognitive impairment, abnormal psychomotor activity and sleep disturbances. Due to high prevalence in hospitalized patients and high rate of morbidity and mortality, delirium significantly decreases the prognosis of hospitalized patients.

Materials and Methods: This experimental animal study evaluated 54 adult male Wistar rats over 2 months of age that weighed 200 to 230g. Of all, 24 rats received biperiden (40mg/kg) while the remaining 24 received 50µg/kg LPS. Induction of delirium was ensured using a Y-maze after 3 hours. The control group included 6 rats The efficacy of different doses of haloperidol for treatment of delirium was assessed 3 h after injection using the Y-maze. Data were compared using one-way ANOVA followed by Tukey's post-hoc test via GraphPad Prism.

Results: Haloperidol at 0.1, 0.2 and 0.5mg/kg dosage significantly increased the percentage of spontaneous alternation and improved the memory, consciousness and learning compared with biperiden and LPS groups ($P<0.01$). No significant difference was noted between the haloperidol groups regarding efficacy ($P>0.05$).

Conclusion: Delirium is characterized by attention deficit, impaired orientation, changes in memory, consciousness, perception and mood, and psychotic symptoms. Detection of the complete spectrum of delirium signs and symptoms in an animal that cannot talk (rat) is obviously difficult. Using of Y-maze can facilitate this problem.

Keywords: Delirium; Y-maze; Biperiden; Haloperidol; Spatial memory.

Introduction

Delirium is a life-threatening, but reversible, syndrome characterized by cognitive impairment, abnormal psychomotor activity and sleep disturbances [1]. The behavioral manifestations of delirium may interfere with the patient's compliance to treatment. Thus, psychological counseling is often required for such

patients [2]. The prevalence of delirium is 14% to 24% in the hospital setting, and increases by prolonged hospitalization (6-56%). Factors such as age, medication intake and associated disorders can increase the prevalence of delirium. Delirium often remains undetected by the medical staff, and the prevalence of its misdiagnosis ranges from 40% to 60%, depending on the study setting [3].

The annual morbidity and mortality rate associated with delirium is 35% to 40%. The causes of delirium may vary and include stress, head trauma, fever, drug toxicity or withdrawal, metabolic disorders and organ insufficiency/failure. Despite several suggested causes, the underlying pathophysiology of delirium has yet to be elucidated. Recent studies suggest neurochemical disorders, inflammatory changes, oxidative stress and blood-brain barrier dysfunction as the possible causes of delirium. Delirium is not often easily diagnosed in medical settings [4]. A precise medical history taken from the patient companions can be of great help. Accordingly, all the causes and predisposing factors should be evaluated. The primary objective in treatment of delirium is to treat/eliminate the underlying cause. Another important objective is to provide physical, emotional and environmental support [4]. It has been witnessed that midazolam is used to manage delirium patients in general wards, intensive care units, cardiac care units, Orthopedic Departments and Emergency Rooms. However, according to the psychiatric text books, administration of midazolam is only indicated to control and treat the symptoms of delirium following alcohol and benzodiazepine withdrawals [5].

Delirium is of lower interest to psychiatrists as a topic of research compared with schizophrenia and depression, and it has been largely overlooked in the emergency rooms and surgical and other medical wards [2]. Since delirium patients are mostly critically ill, they cannot be easily enrolled in clinical trials involving pharmaceutical interventions. Thus, animal studies are designed for this purpose. Studies on delirium in animals are limited. However, the available ones have addressed two possible mechanisms of delirium namely the hypo-cholinergic theory and the systematic inflammation theory [6]. Since the effective mechanisms involved in induction of this syndrome and its treatment can be evaluated in Wistar rats, this study was designed to assess the efficacy of haloperidol for treatment of delirium in rats [7].

Materials and Methods

This experimental animal study evaluated 108 male Wistar rats weighing 200-230g, that were over 2 months of age. The rats were obtained from the animal room of the Neuroscience Research Center of Shahid Beheshti University of Medical Sciences. The animals were kept in cages (4 to 5 rats per cage) under controlled temperature at 24±2°C and 12-h dark/12-h light cycles. The rats had ad libitum access to food and water except for the time of experiment. Each rat was only experimented once. The study was conducted in accordance with the protocols and guidelines for the care and use of laboratory animals by the American National Institute of Health, and the ethical considerations were strictly followed.

Phase 1: According to a pilot study, 42 rats received intraperitoneal injection of biperiden (4mg/kg) while the remaining 42 rats received intraperitoneal injection of 50μg/kg LPS. A control group was also considered, which included 6 rats that received intraperitoneal injection of saline. After the injection of medications, induction of delirium was ensured by behavioral assessment of rats.

Phase 2: In this phase, the Y-maze test was performed 3h after the injection of biperiden and LPS to assess the spatial working memory and learning of rats. Several models are employed to assess the trend of learning and memory of laboratory animal models considering the effect of medications on memory and the major role of hippocampus in spatial memory [8]. A plexiglass Y-maze was used in this study; each arm of the maze measured 15cm (width) x 30cm (height) x 40cm (length). The arms merged at the center (Figure 1) [9].

Figure 1. Y-maze used in this study.

Each rat was placed at the end terminal of each arm and allowed to explore all areas of the maze for an 8-min period. The number of arm entries was recorded for each rat. An entry occurred when all four

limbs were within the arm. The number of arm entries and the number of triads were recorded to calculate the percentage of alternation as follows:

Number of triads/total number of arm entries x 100 [10].

Phase 3: After ensuring the induction of delirium, the rats were randomly divided into 6 groups to assess the therapeutic effect of different doses of haloperidol. The range of dosage was determined based on previous studies regarding the effects of haloperidol on behavior of rats [11,12]. that used a dosage range that did not cause complications (these studies had been conducted on non-delirium cases since no previous study has evaluated the effect of haloperidol on delirium). The medications were obtained from Sobhan Pharmaceuticals and administered via an intraperitoneal injection. Three hours of time was allowed for the medication to take effect, and its effect on memory and learning was evaluated after 3h using the Y-maze.

The nine study groups were as follows:

Placebo group: That received saline injection.

Delirium induced by injection of 50μg/kg LPS.

Delirium induced by injection of 4mg/kg biperiden.

Delirium induced by injection of biperiden and the rats received 0.1mg/kg haloperidol.

Delirium induced by injection of biperiden and the rats received 0.2mg/kg haloperidol.

Delirium induced by injection of biperiden and the rats received 0.5mg/kg haloperidol.

Delirium induced by injection of LPS and the rats received 0.1mg/kg haloperidol.

Delirium induced by injection of LPS and the rats received 0.2mg/kg haloperidol.

Delirium induced by injection of LPS and the rats received 0.5mg/kg haloperidol.

Statistical Analysis

The results were reported as mean±standard deviation. Behavioral data were analyzed using one-way ANOVA followed by Tukey's post-hoc test via GraphPad Prism software. $P<0.05$ was considered statistically significant.

Results

The results of the Y-maze test indicate short-term spatial memory, consciousness and learning of rats. The frequency percentage of spontaneous alternation of adult male rats in the Y-maze test after injection of biperiden and LPS (n=6): The LPS and biperiden groups showed a significant reduction in the frequency percentage of spontaneous alternation compared with the placebo ($P<0.001$, Figure 1). The therapeutic effects of 0.1, 0.2 and 0.5mg/kg doses of haloperidol on the percentage of continuous alternation of adult male rats in the Y-maze test after the injection of biperiden and LPS (n=6) compared with the biperiden and LPS groups are presented in Figures 2 and 3, respectively. The results are presented as mean±standard deviation. *$P<0.005$, **$P<0.001$.

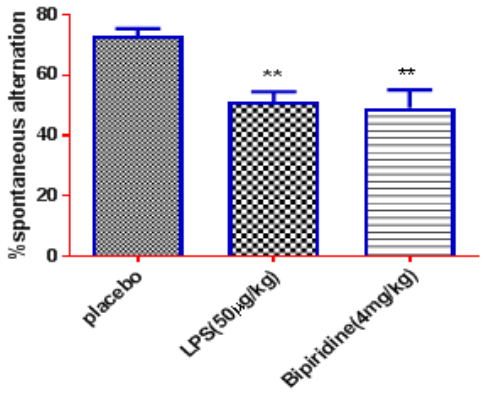

Figure 2. Frequency percentage of alternation in the LPS and biperiden groups compared with the placebo. The results are presented as mean±standard deviation. **$P<0.001$.

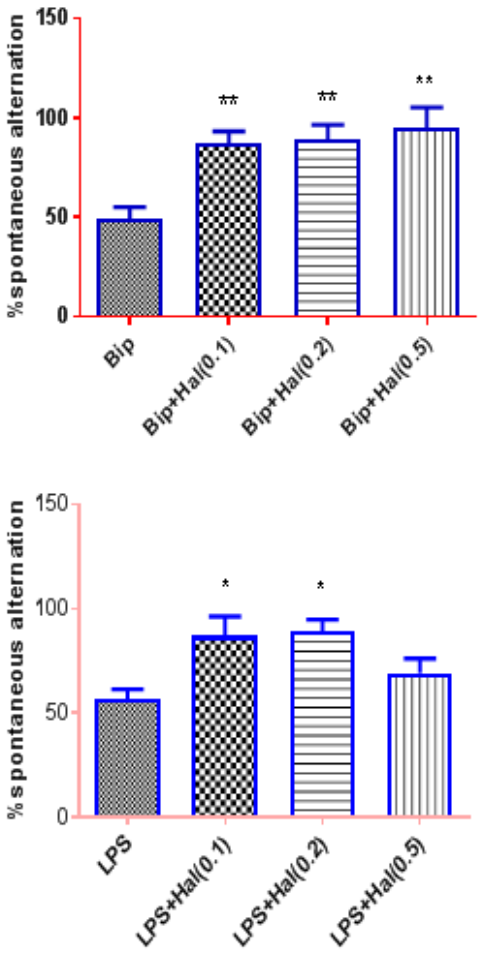

Figure 3.

Discussion

Studies on delirium in animals are limited. However, the available ones have addressed two possible mechanisms of delirium namely the hypo-cholinergic theory and the systematic inflammation theory [6]. Previous studies had addressed one of these mechanisms while in this study, we assessed both mechanisms, which is a strength of our study because we compared the possible causes of delirium as well. According to Cunningham [6] and Maclullich [13], animal models are required to understand the process of development of disease and its treatment in human beings. Induction of delirium was ensured using the Y-maze (learning and memory). Using the anti-cholinergic mechanism, we induced delirium in Wistar rats by intraperitoneal injection of 4mg/kg biperiden according to O'Hare et al, [14] who administered atropine to rats in 1997 and evaluated their behavioral changes using a maze. We used biperiden in our study to induce delirium because it is more commonly used in psychiatric wards. This methodology was in accordance with that of Tamura et al, [15] in 2006 who thoroughly explained the behavioral changes in rats. Also, their study was more recent than others on this theory. Tamura et al. [15] administered 40mg/kg biperiden to Wistar rats to induce delirium and assessed their behavioral changes. We performed a pilot study and showed that use of 4mg/kg biperiden caused the same behavioral changes in rats. However, they could not induce delirium by using 0.4 and 4mg/kg doses in rats. During the course of our study, administration of 4mg/kg biperiden caused significant behavioral changes and decreased spontaneous alternation in rats. Another mechanism used in our study was based on the theory of inflammation induction by using 50µg/kg LPS. Similarly, Cuningham et al, in 2008 used 100µg/kg LPS to induce cognitive changes and neurodegenerative diseases in animal models. Cuningham et al. [16] evaluated the behavioral changes in rats 3h after the injection of LPS using the Y-maze to assess their spatial memory and revealed significant changes. The changes were also significant in our study but in a shorter time, which was closer to the findings of Culley et al, [17] who reported behavioral changes at 2 h after the injection of LPS. Our study was almost similar to that of Cuningham et al, [18] regarding the Y-maze test used for assessment of memory and learning, and evaluation of higher routes involved in development of de-

lirium in the central nervous system. Culley et al, [17] in 2014 reported that 50μg/kg LPS caused systemic inflammation and affected the hippocampal-dependent memory and caused behavioral changes particularly in terms of attention and cognitive flexibility. They used the attentional set shifting task to assess cognitive aging and prefrontal cortex function. Cuningham et al. [16] evaluated the behavioral changes at three different time points: 2 h after injection, 24 h after injection and 48 h after injection. The changes were not significant at 24 and 48 h after injection, and the behaviors returned to normal at 48 h after injection. In our study, LPS caused behavioral changes and spontaneous alternation that lasted for 60 min to 24 h after the injection in case of no treatment and then stopped, which was somehow in line with the results of Cuningham et al [17]. However, in terms of dosage, we induced these changes using a lower dosage of medication.

Induction of delirium via the inflammation mechanism in our study was similar to the studies by Zhipeng et al, [19] in 2014 and Peng et al, [7] in 2016 who induced delirium in rats after a surgical procedure via the inflammation mechanism. In their studies, Y-maze was used to assess the behavioral changes in rats and their learning and spatial memory. In their studies, delirium lasted from 2 h to 9 h after surgery and then subsided and gradually resolved within 24 h after the surgical procedure. Our results were similar to their findings. After the induction of delirium, the therapeutic effect of haloperidol on delirium in adult male rats was evaluated.

No experimental animal study is available on the therapeutic effect of medications on delirium. However, similar studies have been conducted on dementia and Alzheimer's disease in rats. Park et al, in 2017 evaluated the effect of Humulus japonicus on Alzheimer's disease induced in rats [8]. Nakamura et al, in 1998 evaluated the effect of aniracetam on Alzheimer's disease in mice [20]. Javadi-Paydar et al, in 2011 evaluated the effect of atorvastatin [21] and Javadi-Paydar et al, in 2012 evaluated the effect of granisetron on cognitive impairment in mice with induced dementia [22]. In 1998, Nakamura et al. used aniracetam to resolve attention deficit disorder caused by apomorphine [18]. It seems that researchers were more interested to study dementia rather than delirium. The reason may be the uncertainly about the causes of delirium, high number of suggested etiologies and the fact that its treatment is mainly based on the resolution of the underlying pathology.

Studies on treatment of dementia such as the one by Javadi-Paydar et al, in 2012 [22] reported improvement of memory deficit induced by scopolamine using granisetron. Another study by the same authors in 2011 reported improvement of memory deficit induced by scopolamine using atorvastatin. The abovementioned studies used the Y-maze to assess the memory deficit, similar to our study. In our study, the effect of different doses of haloperidol was evaluated on delirium induced by two possible mechanisms. No significant difference was noted between LPS and biperiden groups regarding the therapeutic effect of haloperidol irrespective of the mechanism of induction of delirium. The results of the Y-maze test, which indicates the short-term spatial memory, consciousness and learning of rats, showed no significant difference between delirium induction by LPS and biperiden, and both mechanisms caused significant changes with regard to memory deficit, consciousness and learning of rats.

Haloperidol in lower doses (0.1 and 0.2mg/kg) caused significant improvement in percentage of spontaneous alternation in LPS-induced delirium group compared with 0.5mg/kg dosage. However, this was not the case for biperiden-induced delirium group, and all three doses of haloperidol were effective in this group. The reason may be the fact that haloperidol directly affects the same receptors activated by biperiden (anticholinergic receptors leading to consequent increase in dopamine) and blocks them. However, this is not the case for systemic inflammation, and the extrapyramidal effects gradually appear following the administration of 0.5mg/kg dosage. Thus, we focused on a number of key cognitive and physical symptoms of delirium and used the Y-maze to assess memory deficit and impaired consciousness and learning.

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Conflict of Interest

There is no conflict of interest to declare.

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