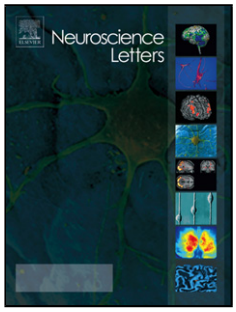

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Protective effects of dopamine D2/D3 receptor agonist piribedil on learning and memory of rats exposed to global cerebral ischemia–reperfusion

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Highlights

- Piribedil has beneficial and harmful effects during global cerebral ischemia-reperfusion injury.
- It could improve activity, memory, and learning capacities of the rates in global cerebral ischemia-reperfusion injury.
- It may be useful clinically for treating severe global cerebral ischemia-reperfusion brain injury.

Abstract: Global cerebral ischemia-reperfusion (GCI/R) may occur after any of several clinical conditions such as cardiac arrest and anesthetic accident. Some dopamine receptor agonists possess neuroprotective effects. However, some of them may produce side effects during treatment.Piribedil, which is a dopamine D2/D3 receptor agonist, has fewer side effects and is well tolerated. This study investigated the effects of

piribedil on learning and memory of rats with GCI/R according to modified neurological severity score (mNSS) scoring and Morris water maze test (MWM). Rats with GCI/R were treated with piribedil 25 or 50 mg/kg/d, and mNSS was performed at 6 hours, 1 day, 3 days, and 1 and 2 weeks after injury. The MWM test was employed to evaluate learning and memory of rats at 1 and 2 weeks after injury. The results showed treatment with piribedil reduced the mNSS score and prolonged the time in the target quadrant compared with untreated rats although no obvious differences of the 25 and 50 mg/kg/d piribedil intervention groups were observed statistically. Piribedil is effective in improving the neurological function and learning and memory of rats after GCI/R.

Abbreviations: DA, dopamine; GCI/R, global cerebral ischemia/reperfusion model; PD, Parkinson disease; mNSS, modified neurological severity score; H&E, hematoxylin and eosin; 4-VO, four-vessel occlusion.

Keywords: Piribedil, Cerebral ischemia-reperfusion injury, Learning memory, Behavior, Morris water maze.

Introduction

Global cerebral ischemia may occur in different clinical situations such as cardiac arrest, shock, asphyxia, and major cardiac surgery, and patients usually develop secondary brain injury after successful resuscitation from these conditions [10, 34]. Currently, global cerebral ischemia–reperfusion (GCI/R) is one of the major causes of brain injury under these conditions [24]. After cardiac arrest, more than 44% of patients can be successfully resuscitated, but 80% of these will remain in a persistent vegetable state or will die, and remaining patients may develop neurologic dysfunctions that affect consciousness, language, cognition, and motor functions [15].

Four dopaminergic nerve pathways are present in the central nervous system (CNS): the nigrostriatal pathway, the mesolimbic pathway, the mesocortical pathway and tuberoinfundibular pathway. Different brain regions, e.g. the midbrain, striatum, amygdala, hippocampus, prefrontal cortex and hypothalamus are connected through these pathways [1, 13], which are closely related to behavioral events, including reward processing, spatial learning, and work memory[2]. Of these regions, the hippocampus is a major region

responsible for long-term learning and memory of events (sound, light, and taste, as well as narrative memory), and place cells and grid cells are also localized in these regions [17, 27].

Dopaminergic projections originate primarily from the midbrain, e.g. the ventral tegmental area (VTA), substantia nigra, and retrorubral field [22, 23]. The locus coeruleus also project dopamine directly to the hippocampus[22]. Dopamine secreted in the midbrain and locus coeruleus may be transmitted to the hippocampus through dopaminergic fibers, where it activates pyramidal cell to promote the reactivation of newly encoded hippocampal representations and improve memory performance[5, 23]. However, both the midbrain and limbic system are sensitive to ischemia and hypoxia, especially the hippocampus that related to advanced learning and memory [13]. The death of dopaminergic neurons in the midbrain leads to an imbalance between the release and synthesis of dopamine, and the altered transmission of dopamine to the hippocampus may be important for causing motor and cognitive impairments in survivors after GCI/R[6].

Our previous studies revealed that reoxygenation and low-dose levodopa-dopamine can protect the brain against GCI/R in rats, improve their learning and memory [30, 31], and reduce the death of hippocampal neurons in mice [32]. However, evidence shows that long-term use of levodopa may cause motor impairment [20]. Dopamine receptor agonists can inhibit or prevent motor impairment [3]. The addition of dopamine D2/D3 receptor agonists may improve storage and maintenance of memory [7] and reduce the glutamate

oxidative stress in oligodendrocytes that experience glucose and oxygen deprivation. In addition, past research showed that dopamine receptor agonists (such as bromocriptine, pergolide, and lisuride) were protective of the hippocampal neurons in the CA1 region [26]. However, not all of the dopamine receptor agonists with neuroprotective effect are suitable for the GCI/R treatment, and some of them may have side effects [4].

Five subtypes of dopamine receptor have been identified: D1–D5 receptors, and they can be divided into 2 groups: ergot-derived and non-ergot-derived dopamine agonists. Bromocriptine, pergolide, risumergine, methanesulfonic acid a^3-dihydroergocryptine, and cabergoline belong to the ergot-derived dopamine agonists. The non-ergot-derived dopamine agonists comprise piribedil and pramipexole. Some of them, such as bromocriptine and pergolide, raise risks for patients who have a history of cardiovascular diseases, psychosis, and depression [4]. Piribedil has certain side effects such as nausea, vomiting, dizziness, confusion. However, the non-ergot-derived dopamine agonist piribedil has fewer side effects and is well tolerated [4].

To date, few drugs have been developed for early neuroprotection in brain resuscitation, and none has confirmed neuroprotection according to the results of clinical studies. On the basis of previous findings, the present study investigated the effects of the dopamine D2/D3 receptor agonist piribedil on memory, learning, and hippocampal neurons of rats with GCI/R on the basis of the modified neurological severity score (mNSS) and Morris water maze test.

This study investigated whether piribedil provides early neuroprotection that can improve neurological function after brain injury in rats.

1. Experimental methods

1.1. Subjects

We used adult male Sprague-Dawley rats (weight 300 ± 20g) obtained from the Experimental Animal Center, Academy of Military Medical Sciences, Beijing, China. All animal procedures used in the present study were approved by the Institutional Animal Care and Use Committee of Capital Medical University (IACUC: AEEI-2015-138).

Fifty-six rats were randomly divided into 4 groups, 8 in the sham-operated group, and 16 animals in each of other three groups namely model group, one week and two weeks group, the 16 animals in each group were further divided equally into a low and high-dose (25 mg/kg/d or 50 mg/kg/d Piribedil) subgroups.

1.2. The GCI/R model

The modified four-vessel occlusion (4-VO) method introduced *by Pulsinelli* et al. was used as described by our study previously[31]. After 24-hour fasting, rats were injected intraperitoneally with 40mg/kg Nembutal for anesthetization. The bilateral vertebral arteries were coagulated occluded with electro-coagulation. Twenty-four hours later, 40mg/kg Nembutal was again injected intraperitoneally to anaesthetize the rats. Noninvasive vascular clamps were used to clip the bilateral common carotid arteries for 10 minutes (ischemia stage) and were removed 10 minutes later (perfusion stage). The same operation was performed in the two Piribedil intervention groups. Meanwhile, the sham operation was performed as per

the normal surgical exposure procedures except of occlusion to the vertebral arteries and the carotid artery.

1.3. Administration of drug

After the GCI/R model was established, intragastric administration of 25 mg/kg/d and 50 mg/kg/d Piribedil was performed for each rat in the Piribedil intervention groups immediately. Piribedil (20 mL: 50 mg/vial) was from Les Laboratoires Servier (France). Normal saline of 25 mg/kg/d was intragastrically injected into the rats of the sham-operated and GCI/R model groups. The stomach feeding on each rat is continued for 7 days.

1.4. The modified neurological severity score (mNSS)

The mNSS is a composite for evaluating motion, sensory, reflex, and balance capacities, which is widely used in animal studies of brain injury as one of the most common neurological scales. The mNSS of each rat was scored and recorded before operation and 6 hours, 1 day, 3 days, and 1 and 2 weeks after reperfusion. Neurological function was graded on a scale of 0 to 18. 0 indicates normal, 1 to 6 points is a mild injury, 7 to 12 points is a moderate injury, and 13 to 18 points is a severe injury. A double-blind method was used in this study. In other words, the results were independently observed by two investigators, and the average value of results was recorded.

1.5. Morris water maze (MWM)

The abilities of learning and memory were measured using MWM (Hong Kong Biotechnology Co., Ltd.). The procedure was modified according to the Patel AD's method [23]. The MWM comprises a place navigation test and a spatial probe trial, which were conducted in the department of experiment, Capital Medical University.

The MWM test was carried out 1 or 2 weeks after GCI/R injury. A large circular pool with a diameter of 1.5m was used as the apparatus, which was filled with water at 23 ± 1 °C. The pool was separated into 4 parts (I, II, III, IV) with visual cues on the walls for navigation. A platform was located in the II zone.

The place navigation test was conducted by means of four trials daily for 4 consecutive days. For each trial, rats were placed in the water facing the wall of the pool but at different start positions between trials. Each rat was trained to find the platform for a maximum of 60 s. The time taken by rats to find and climb on the platform (escape latency) was observed and recorded, as well as the tendency of changes in linear search strategy.

The spatial probe trial was started on the fifth day. The platform was removed and behavior of the mice was recorded for 60 s.

2.5. Statistical analysis

All results were presented as mean±SD and analyzed by the SPSS program, version 17.0 statistic software package (SPSS Inc. Chiacago, Illinois, USA). The weight of each rat was analyzed by one-way analysis of variance (ANOVA). The escape latency was analyzed by repeated measures of ANOVA. Spearman's rank correlation test was used for analyzing the consistency of the mNSS. The daily escape latency, swimming speed and percentage of time spent in the second quadrant were analyzed by multivariate analysis of variance. Post hoc analysis was performed using LSD-t test.

3. Results

3.1. mNSS Test

The mNSS was 0 in all groups before operation. In the sham-operated group, the mNSS was 0 at 6 hours, 1 day, 3 days, 1 week and 2 weeks after reperfusion (Table 1). The mNSS score was analyzed by multivariate analysis of variance followed by post-hoc Games–Howell test. The score at 6 h after reperfusion was the highest and thereafter gradually decreased (Table 1) in all experimental groups. After reperfusion, the mNSS was 0 in all groups. At 6 h after reperfusion, no statistics difference of mNSS was found among the sham-operated group, the 25 mg/kg/d, and 50 mg/kg/d piribedil intervention groups (Table 1 and Figure 1). At 24 h after reperfusion, the mNSS score of the rats was lower in the 25 mg/kg/d and 50 mg/kg/d piribedil intervention groups compared with the GCI/R model group ($P < 0.01$) (Table 1 and Figure 1). There were no significant differences in statistics between the two

groups that received piribedil. At 72 h and 1 w after reperfusion, the score in the 50 mg/kg/d piribedil intervention group was the lowest among the two groups of piribedil-treated rats and the sham-operated group ($P < 0.05$) (Table 1 and Figure 1). The mNSS score of rats in the 25 mg/kg/d piribedil intervention group was similar to that of rats the GCI/R model group. The mNSS score had no statistics difference among the groups two weeks after reperfusion (Table 1 and Figure 1).

3.2. Morris water maze test

During the Morris water maze test, we observed and recorded the swimming paths of the rates in each group. The swimming paths of the rats in the sham-operated group were smoother compared to the other three groups (Figure 2).

The escape latency of all groups showed a downward trend (Figures 3A and 4). The GCI/R model group showed marked retardation in their escapes (Figures 3 and 4). At one week after reperfusion, the daily escape latency was analyzed by multivariate analysis of variance. The results showed the daily escape latency was a significant difference in statistics among groups ($F=12.688$, $P < 0.01$). The latency in the GCI/R model group was longer than that in the other three groups ($P < 0.05$). In latency, the 25 mg/kg/d and 50 mg/kg/d piribedil intervention groups had no significant difference in statistics, although the latencies of the two groups were significantly shorter than those in the GCI/R model group ($P < 0.01$) (Figure 3B).

At two weeks after reperfusion, although the mean latencies of two piribedil intervention groups were similar to that of rats the GCI/R model group, the latency in the 25 mg/kg/d piribedil intervention group on the third day was shorter than that in the GCI/R model group (F=4.234, $P < 0.01$), and the latencies of the two piribedil intervention groups on the fourth day were significantly shorter than that in the GCI/R model group (F=7.350, $P < 0.05$).

During the probe trial, multivariate analysis of variance followed by a post-hoc LSD test was used for analyzing the swimming speed and the percentage of time spent in the second quadrant. The swimming speed and the time spent in the second quadrant had no statistics difference between one and two weeks after reperfusions. The time spent in the second quadrant had significant differences among groups (at one week after reperfusions F=31.495, $P < 0.01$); and at two weeks after reperfusions (F=28.790, $P < 0.01$). The time spent in the second quadrant was shorter in the GCI/R model group compared to the other three groups (P<0.05) (Figures 5A and 5B). The time spent in the second quadrant was longer in the two piribeidl intervention groups compared with the GCI/R model group (P<0.05) (Figures 5A and 5B). The mean swimming speed or escape latency during the visible platform test were no significant differences among the various groups.

4. Discussion

This study showed that dopamine D2/D3 receptor agonist piribedil restored the impairment of working memory caused by the GCI/R injury. Dopamine D2 receptors are expressed in the

VTA, substantia nigra, striatum and hippocampus[9, 14]. D3 receptors are also found in hippocampus, septum, or mammillary nuclei of the hypothalamus[14]. Activation of dopaminergic D2/D3 receptors repaired dorsoventral connectivity of the hippocampus and working memory after nerve injury [6]. Additionally, administration of the dopamine D2/D3 receptor agonist may increase dopamine in the rat brain [8, 12].

Previous studies have shown that piribedil, traditionally used in the treatment of Parkinson's disease, can mitigate consciousness disorders in patients with GCI/R[18]. Our results showed that piribedil can improve neurological function, learning, and memory in rats with GCI/R. These results are consistent with the findings in available studies.

GCI/R may cause damage to the neurons of the CNS, including early cell necrosis and delayed neuronal death[35], especially cells in the regions related to advanced learning and memory (such as the hippocampus, striatum, and amygdala). GCI/R may cause a disruption of dopaminergic nerve pathways, leading to a reduction in dopamine secretion. Thus, the addition of dopamine to prime the dopamine pathway may improve patients' cognition. Piribedil can activate dopamine D2/D3 receptors in VTA and hippocampus[14], result in an increasing of the intracranial dopamine concentration and restoring the normal dopamine transmission system[12].

Previous studies have shown that dopamine supplement with levodopa improves the symptoms of aphasia, learning, and the motor function of patients after stroke [11] and also is

beneficial for the improvement of consciousness disorders after GCI/R [16, 19, 29]. In vivo experiments revealed that levodopa may improve both the learning and memory in rats after GCI/R [32], but the long-term use of levodopa or high-dose levodopa may cause certain side effects[3]. In the treatment of Parkinson disease, some studies indicate that levodopa is effective for treating PD symptoms, but long-term or high-dose use of levodopa can reduce therapeutic efficacy or induce a “wearing-off phenomenon,” an “on–off phenomenon,” and other untoward complications [3]. Thus, a dopamine receptor agonist was used in this study for continuous dopaminergic stimulation and the reduction of side effects [3].

Dopamine D2/D3 receptor agonists can not only continuously stimulate dopamine receptors to increase intracellular dopamine [8] and improve dopamine storage and maintenance of memory [7], but they also can reduce or avoid the fluctuation of symptoms or the occurrence of motor complications [3]. Compared with levodopa, piribedil has a longer half-life and may ensure a more stable plasma concentration and continuous dopaminergic stimulation without producing oxygen free radicals [28]. In vivo studies indicate that piribedil can regulate dorsoventral connectivity in the hippocampus [7] and improve aging-induced damage to memory[21]. The dopamine D2 receptor in the hippocampus is responsible mainly for regulation of long-term potentiation and long-term depression, for example, in learning and memory [25]. After remodeling, dopaminergic neurons may re-activate the hippocampus and improve spatial memory [23]. Our results showed that piribedil treatment can improve escape

latency and working memory as compared with untreated rats. These findings are consistent with published studies.

The Morris water maze test showed that piribedil can significantly improve advanced cerebral functions such as learning and memory after GCI/R. However, at one week and two weeks after GCI/R, there was no significant difference in escape latency between treated rats and untreated rats, suggesting that learning does not improve or attenuate over time within two weeks. This might be ascribed to the proximity between the two time points studied, which failed to identify longer-term time-dependent effects. In addition, to investigate whether motor dysfunction affects swimming posture and speed, we assessed the swimming speed of rats in 4 groups and observed no significant differences. The visualized platform test also revealed the absence of marked differences in the escape latency, suggesting that there was no difference in the vision of these rats. These findings may exclude a false prolongation of escape latency due to motor and vision disorders.

Piribedil is not the only dopamine receptor agonist that possesses neuroprotective effects, and neuroprotection has also been confirmed following treatments with bromocriptine, pergolide, and lisuride [26]. The D2/D3 receptor agonist pramipexole may promote the proliferation and survival of the neuronal cells [33]. However, an in vivo experiment showed that, compared with bromocriptine, piribedil can improve aging-induced damage to memory in rats[21]. The neuroprotection of dopamine receptor agonists and their anti-oxidative effects are related to

the regulation of intracellular dopamine concentrations [26]. We speculate that piribedil may alter the extracellular dopamine concentration to balance the dopamine concentration inside and outside of cells in order to induce neuronal repair. Our future studies will focus on whether the therapeutic effect of piribedil is related to intracerebral dopamine concentration, repair, and reconstruction of the dopaminergic system, and promote anti-oxidative activity by detecting hippocampal dopamine concentrations and oxidation-related parameters, which may elucidate the mechanisms underlying the neuroprotection of piribedil on GCI/R.

In summary, the dopamine D2/D3 receptor agonist piribedil is effective in improving neurological function, learning, and memory after GCI/R in rats, but the specific mechanism requires further study.

Conflict of interest

The authors declare no conflict of interest.

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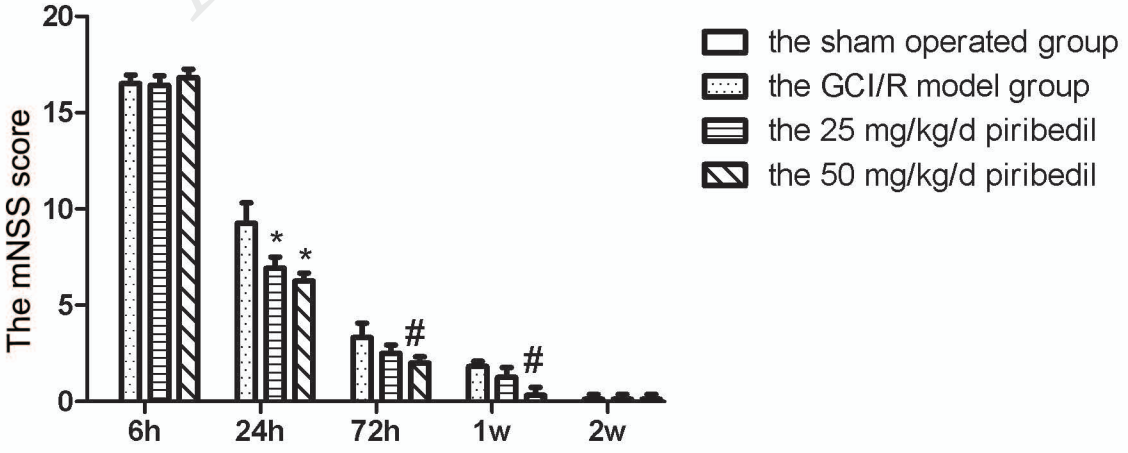
Fig. 1. The mNSS score of the rats during 2 weeks after ischemia-reperfusion injury in each group. *P < 0.05 compared with the sham-operated group. #P < 0.05 compared with the GCI/R model group. Error bars represent standard deviation of the mean (mean ± SD).

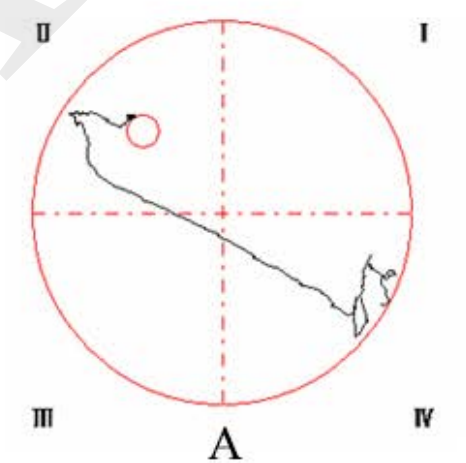
Fig. 2. Swimming paths of the rates in each group during the Morris water maze test. A. the sham operated group. B. the GCI/R model group at 1 week after reperfusion. C. the 25 mg/kg/d piribedil intervention group at 1 week after reperfusion. D. the 50 mg/kg/d piribedil intervention group at 1 week after reperfusion. E. the GCI/R model group at 2 weeks after reperfusion. F. the 25 mg/kg/d piribedil intervention group at 2 weeks after reperfusion. G. the 50 mg/kg/d piribedil intervention group at 2 weeks after reperfusion.

Fig. 3. A. The trend of the escape latency of all groups 1 week after reperfusion. B. Comparison the mean value of escape latency of three daily trail among groups 1 week after reperfusion. ##P < 0.01 compared with the GCI/R model group. Error bars represent standard deviation f the mean (mean ± SD).

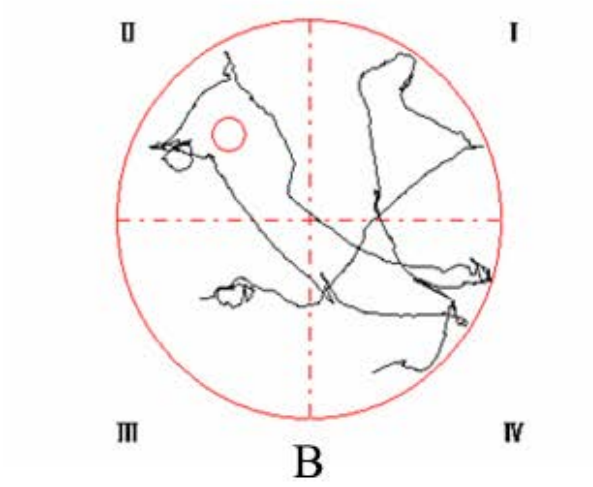
Fig. 4. The trend of the escape latency of all groups 2 weeks after reperfusion. Error bars represent standard deviation f the mean (mean ± SD).

Fig. 5. The percentage of time spent in current goal quadrant 1 week after reperfusion (A) and 2 weeks after reperfusion (B). ##P < 0.01 compared with the GCI/R model group. Error bars represent standard deviation of the mean (mean ± SD).

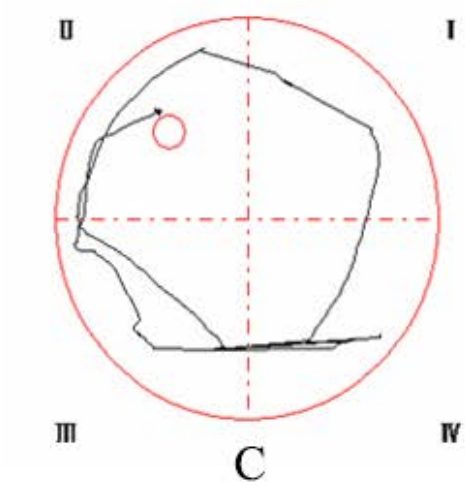

20
15
10
5
0
The mNSS score
6h
24h
72h
1w
2w
*
*
#
#
the sham operated group
the GCI/R model group
the 25 mg/kg/d piribedil
the 50 mg/kg/d piribedil


II
I
III
IV

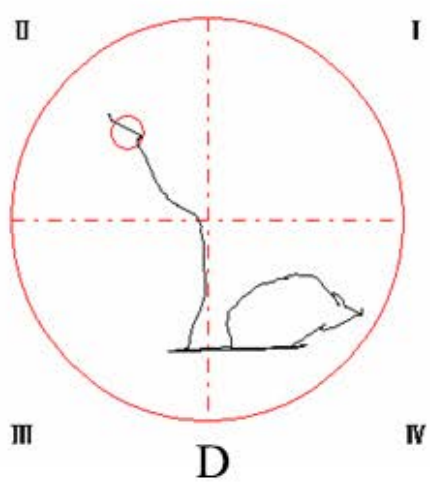
A


II
I
III
IV

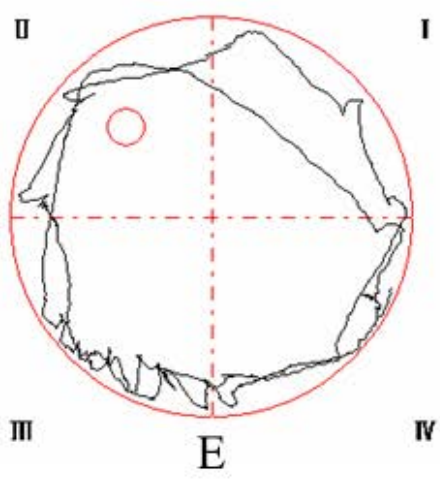
B


II
I
III
IV

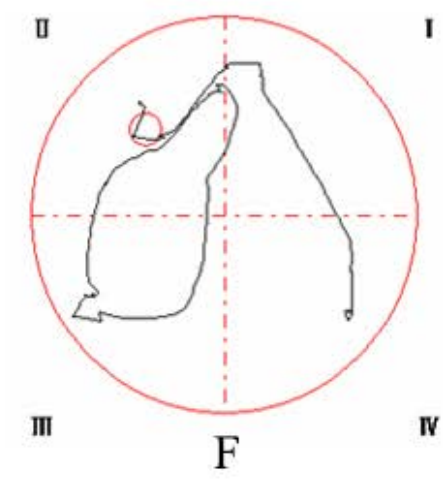
C


II
I
III
IV

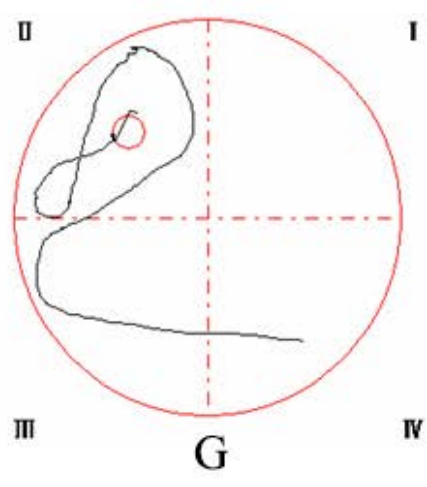
D


II
I
III
IV

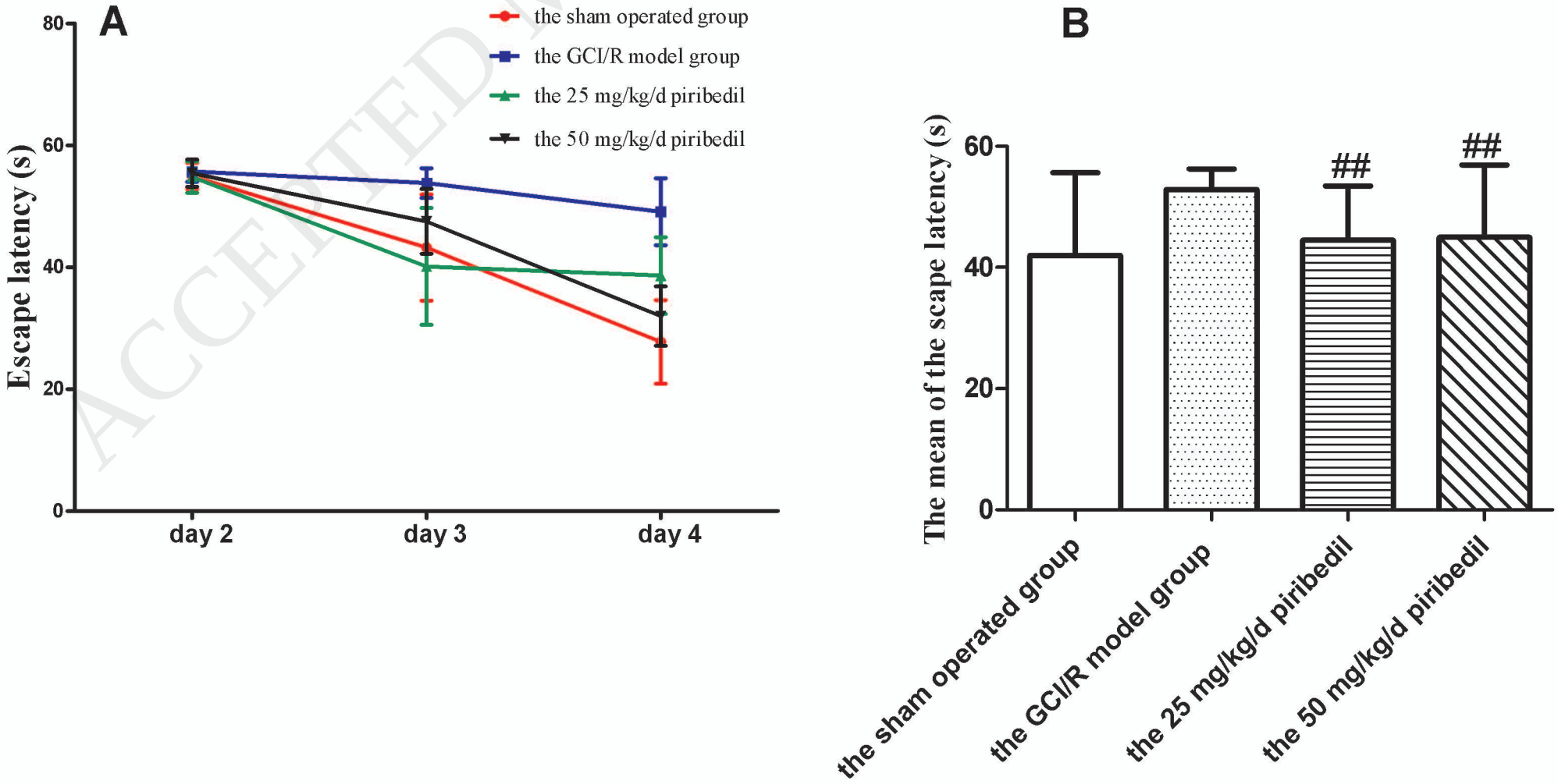
E

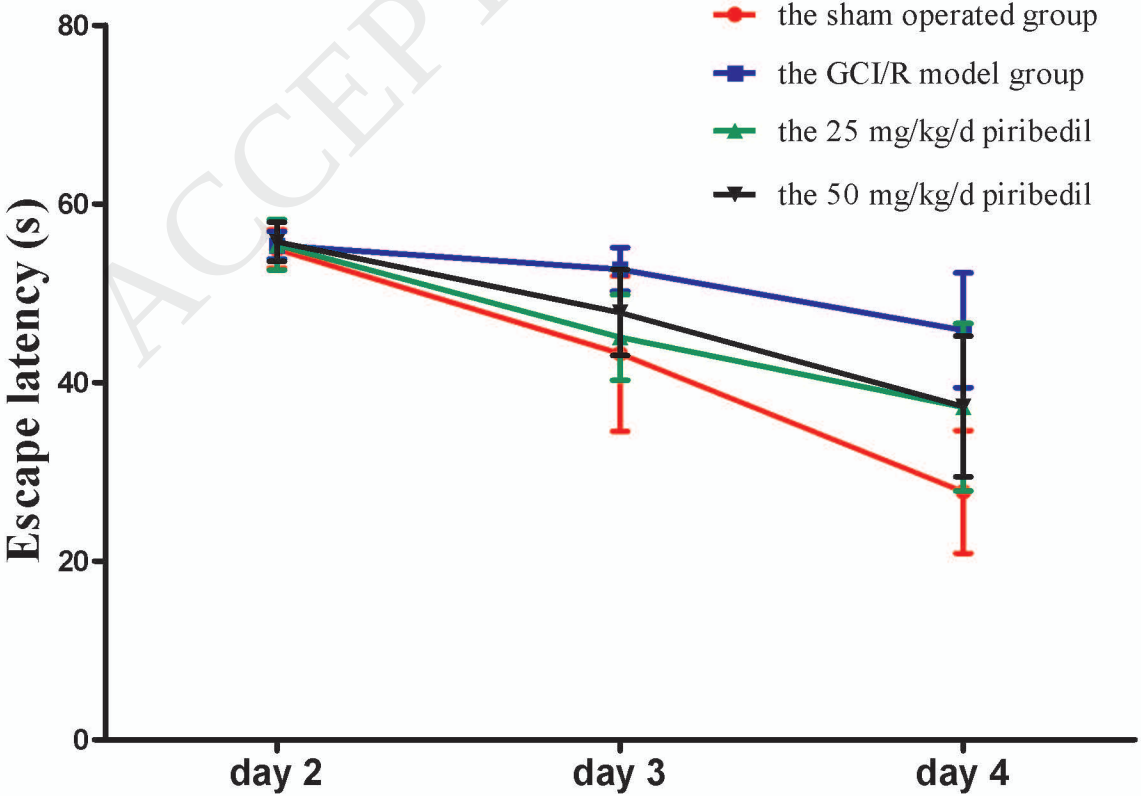

II
I
III
IV

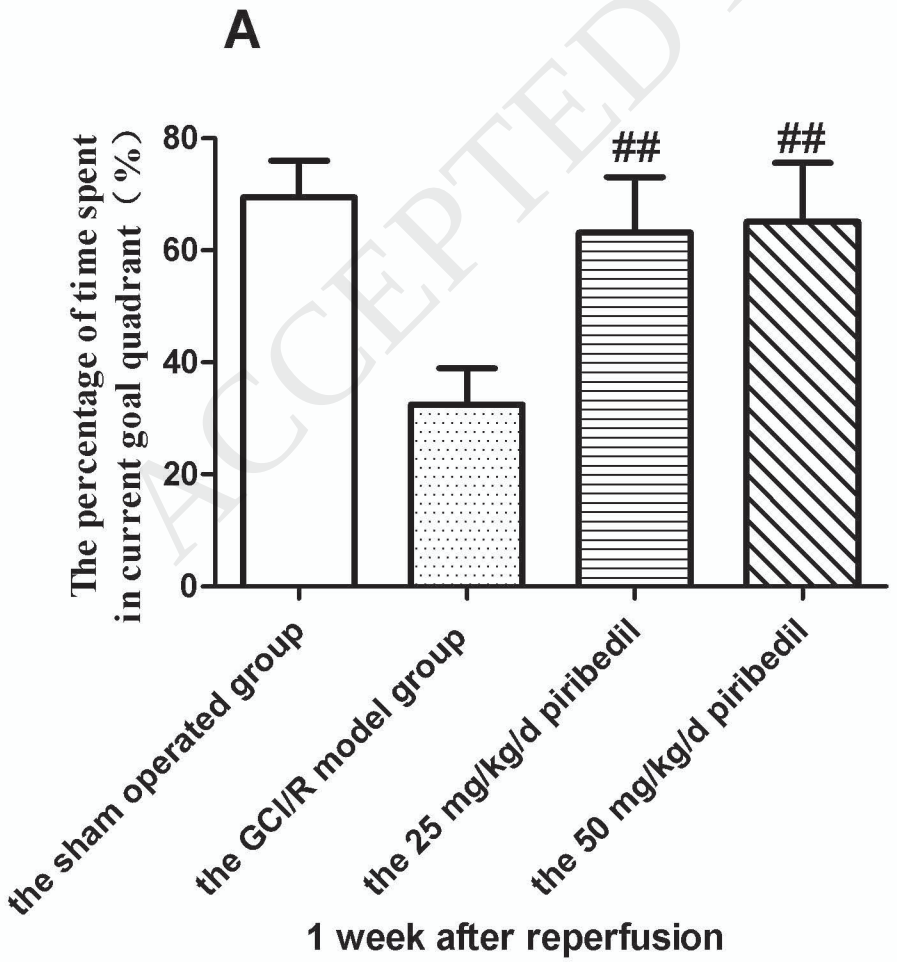
F


II
I
III
IV

G


A
the sham operated group
the GCI/R model group
the 25 mg/kg/d piribedil
the 50 mg/kg/d piribedil
Escape latency (s)
80
60
40
20
0
day 2
day 3
day 4
B
The mean of the scape latency (s)
60
40
20
0
##
##
the sham operated group
the GCI/R model group
the 25 mg/kg/d piribedil
the 50 mg/kg/d piribedil


the sham operated group
the GCI/R model group
the 25 mg/kg/d piribedil
the 50 mg/kg/d piribedil
Escape latency (s)
80
60
40
20
0
day 2
day 3
day 4


A
The percentage of time spent in current goal quadrant（%）
80
60
40
20
0
##
##
the sham operated group
the GCI/R model group
the 25 mg/kg/d piribedil
the 50 mg/kg/d piribedil
1 week after reperfusion

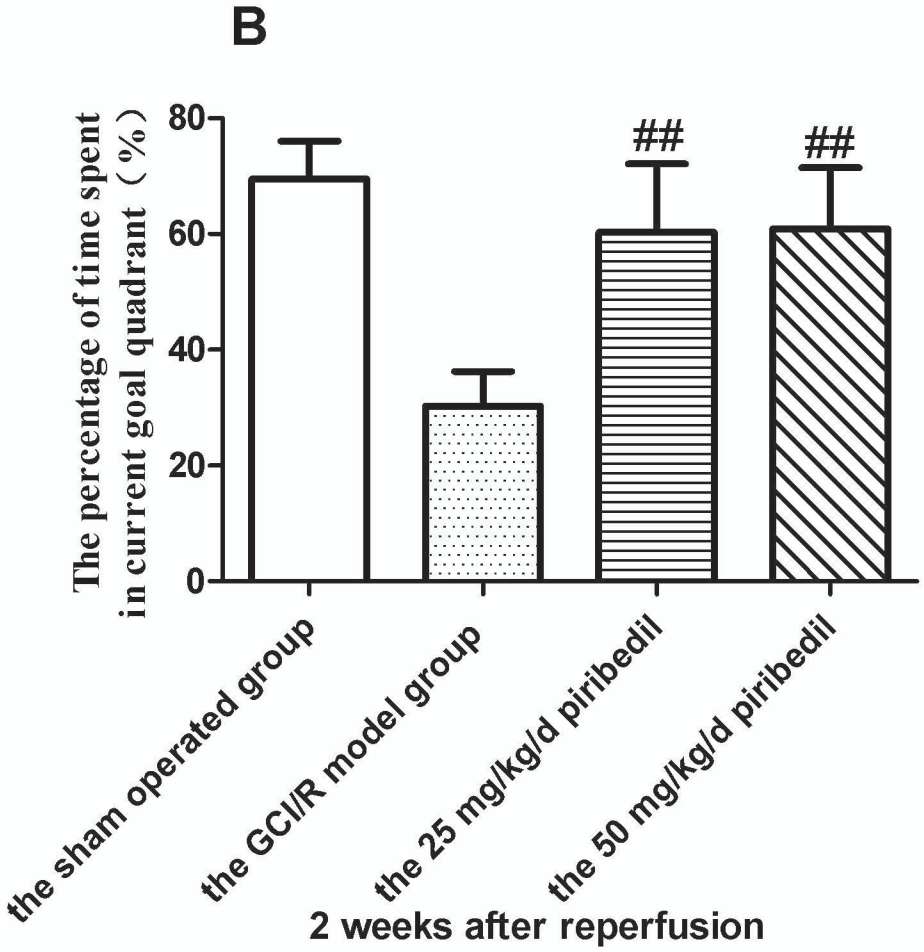

B
The percentage of time spent in current goal quadrant（%）
80
60
40
20
0
##
##
the sham operated group
the GCI/R model group
the 25 mg/kg/d piribedil
the 50 mg/kg/d piribedil
2 weeks after reperfusion

Table 1. The mNSS analysis to detect the difference in functional recovery among groups.

Global Test of mNSS						
Group	n	6h	24h	72h	1w	2w
sham-operated	8	0	0	0	0	0
GCI/R model	16	16.50±0.45	9.25±1.08	3.33±0.75	1.83±0.26	0.13±0.25
the 25 mg/kg/d	16	16.42±0.49	6.92±0.58	2.50±0.44	1.25±0.52	0.13±0.25
the 50 mg/kg/d	16	16.83±0.42	6.25±0.42	2.00±0.32	0.33±0.41	0.13±0.25
F		2331	150.744	52.795	16.463	0.333
p		0.000	0.000	0.000	0.000	0.802