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Early treadmill training promotes motor function after hemorrhagic stroke in rats

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ABSTRACT

Rehabilitation after a stroke is very important because it has beneficial effects on brain function, including the promotion of plasticity. However, an optimal time window for rehabilitation interventions after hemorrhagic stroke has not been clearly defined. The aim of this study was to determine whether early exercise training initiated 24 h after an intracerebral hemorrhage (ICH) might enhance neurologic recovery more than exercise initiated 1 week after ICH without hematoma expansion and edema volume increase. We subjected adult male Sprague–Dawley rats to experimental ICH by the intrastriatal administration of bacterial collagenase. The rats were randomly divided into the following 2 groups: early training group (treadmill exercise started 24 h post-ICH; $n = 18$) and late training group (treadmill exercise started 1-week post-ICH; $n = 18$). Two weeks after surgery we performed neurologic tests (rota-rod, modified limb-placing, and adhesive-dot removal tests), and measured hematoma volumes and brain water content. In the late training group, compared with the pre-ICH performance on the rota-rod test (98.3 ± 69.4 s), the animals had significantly worse performance after the post-ICH rehabilitation (40.5 ± 52.6 s; $p < 0.01$, paired t-test). In the early training group however, the motor performance after the post-ICH rehabilitation (56.4 ± 73.5 s) was not significantly different from the baseline pre-ICH performance (79.8 ± 33.9 s; $p = 0.24$). There were no significant differences between the two groups with respect to the other neurologic tests. Early exercise did not increase hematoma size or brain water content. Early treadmill training could be performed safely, and enhanced motor recovery in a rat model of ICH. Further studies are required to translate the results into clinical significance.

Spontaneous intracerebral hemorrhage (ICH) is a major public health problem with an annual incidence of 10–30 per 100,000 population [15,24], accounting for 2 million (10–15%) of 15 million strokes worldwide each year [27]. The mortality of ICH is higher than that of ischemic stroke (34% at 3 months) [7]. Early mobilization and rehabilitation are recommended in patients with acute stroke who are clinically stable after acute management. However, an optimal time window for rehabilitation interventions after hemorrhagic stroke has not been clearly defined [6].

In a rat model of focal brain ischemia, the early initiation of exercise increases cortical tissue loss and significantly interferes with restoration of motor function [11,25]. In contrast, a study has shown that early exercise training can significantly reduce brain infarct volume and improve neurologic outcomes in rats, when compared with spontaneous recovery [28]. Few studies have been conducted to determine an optimal time window for the initiation of rehabilitation after ICH [18].

Because of the concerns for possible neurologic deterioration due to hematoma expansion after exercise, rehabilitation is not routinely performed during the acute period of an ICH. We hypothesized that (1) early exercise training, initiated 24 h after an ICH, could enhance neurologic recovery more than exercise initiated 1 week after ICH and (2) early exercise training might not expand hematoma volume and increase brain edema. We used a rat model of experimental ICH, induced by intrastriatal administration of bacterial collagenase, and applied treadmill training to the animals.

Thirty-six male Sprague–Dawley rats weighing 240–280 g were used in these experiments. All experimental procedures were approved by the Care of Experimental Animals Committee of University Hospital. The rats were subjected to experimental ICH by intrastriatal administration of bacterial collagenase type VII and randomly divided into the following two groups: an early training group (treadmill exercise started 24 h post-ICH; $n = 18$); and a late training group (treadmill exercise started 1-week post-ICH;

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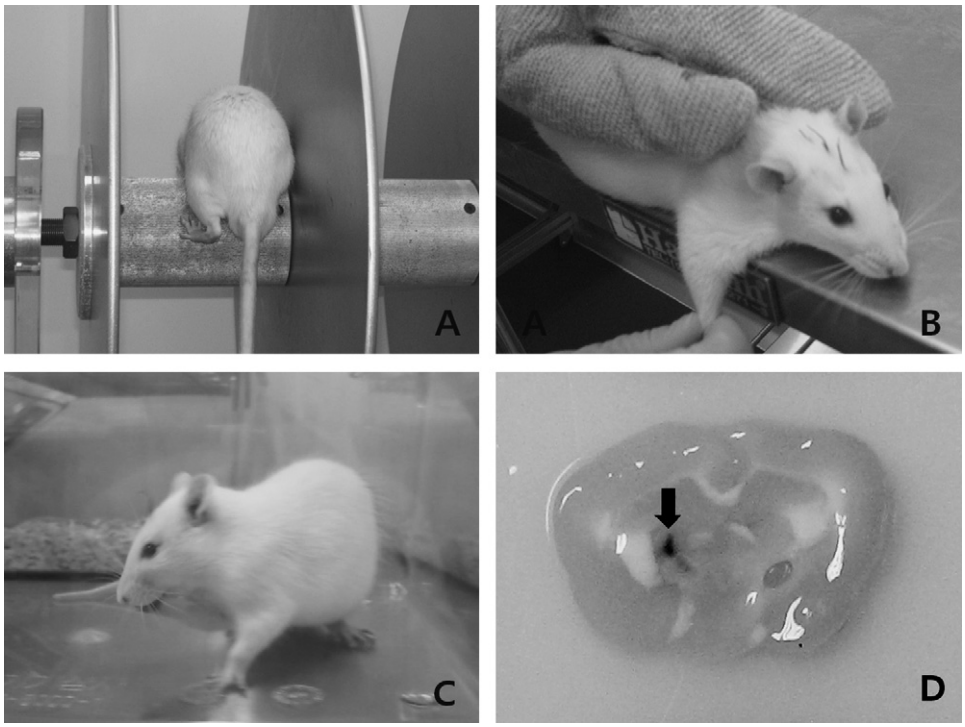

Fig. 1. (A) Rota-rod test. The time the rat remained on the rota-rod is measured. (B) Modified limb-placing test. Stretch, retrieval, and placement of the forelimb are checked while the rat is suspended 10 cm over the table, positioned along the edge of the table, and placed toward the table. (C) Adhesive-dot removal test. Circular adhesive papers are affixed to the forelimb. The latency to remove the papers with the mouth is recorded. (D) Brain section after 2-week training. The arrow shows deep cerebral hemorrhage.

$n = 18$). Two weeks after surgery, we performed neurologic tests (rota-rod, modified limb-placing, and adhesive-dot removal tests), and measured hematoma volumes and brain water content.

An ICH was induced as published before [12] by stereotaxic, intrastriatal administration of bacterial collagenase. After an intraperitoneal injection of 1% ketamine (30 mg/kg) and xylazine hydrochloride (4 mg/kg), the rats were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). A burr hole was made, and a 30-gauge Hamilton syringe needle was inserted through the burr hole into the striatum (coordinates: 0.2 mm posterior, 6.0 mm ventral, and 3.0 mm lateral to the bregma). An ICH was induced by the administration 1 μL containing 0.23 collagen digestion unit (CDU) of collagenase type VII (Sigma, St. Louis, MO, USA) over 5 min. After completion of a collagenase infusion, the craniotomies were sealed with bone wax, wounds were closed with sutures, and the rats were allowed to recover. The entire surgical procedure typically lasted 5–10 min.

During the recovery period, rats were assessed for forelimb flexion and contralateral circling to confirm the procedures. No cases of seizures were observed during the experiments at any time following the procedure. The rectal temperature was maintained at $37 \pm 0.5\,^{\circ}C$ using a thermistor-controlled heating blanket. Free access to food and water was allowed after recovery from anesthesia. Rats were kept in air-ventilated cages at $24 \pm 0.5\,^{\circ}C$ for the duration of the experiment.

A motor-driven treadmill (DJ344; Deajong Instrument INC, Seoul, Korea) was used for the training protocol. Before the ICH procedure, all rats were placed on a moving belt facing away from the electrified grid and run in the direction opposite of the movement of the belt over a 3-day accommodation period. Rats in the early training group were scheduled to start exercise on post-operative day (POD) 1, and rats in the late training group were scheduled to start running on POD 8. The treadmill training was 30 min per day (5 days a week) with a speed range from 2 to 20 m/min and a 0° slope.

Two weeks after surgery we performed neurologic tests (rota-rod, modified limb-placing, and adhesive-dot removal tests). First, rats were placed on the rota-rod cylinder, and the time the animals remained on the rota-rod (Fig. 1A) was measured. The speed was slowly increased from 4 to 40 rpm within a period of 5 min. The trial was ended if the animal fell off the rungs or gripped the device and spun around for two consecutive revolutions [3]. The animals were trained for 3 days before the stereotaxic operation. The maximum duration (in seconds) on the device was recorded with three rota-rod measurements 1 day before ICH induction. The modified limb-placing test (Fig. 1B) consists of two limb-placing tasks that assess the sensorimotor integration of the forelimb and the hindlimb by checking responses to tactile and proprioceptive stimulation [23]. The rat was suspended 10 cm over a table, and the stretch of the forelimbs toward the table was observed and evaluated (normal stretch, 0 points; abnormal flexion, 1 point). Next, the rat was positioned along the edge of the table, with its forelimbs suspended over the edge and allowed to move freely. Each forelimb (forelimb, second task; hindlimb, third task) was gently pulled down, and retrieval and placement were checked. Finally, the rat was placed toward the table edge to check for lateral placement of the forelimb. The three tasks were scored in the following manner: normal performance, 0 points; performance with a delay (2 s) and/or incomplete, 1 point; and no performance, 2 points. A total of 7 points indicated a maximal neurologic deficit, and 0 points indicated a normal performance. Adhesive-dot removal test (Fig. 1C) was performed. A circular adhesive paper (Post-it® Small Flags, size -0.472×1.719 in.; 3 M, St. Paul, MN, USA) was affixed to the distal radius area of the forelimb. The forepaw was held apart and away from the animal's mouth while the rat was returned to its home cage. The latencies to remove the stimuli with the mouth were recorded for the stimuli on the forelimb. The maximum cutoff latency was 3 min, and rats received three trials per session, with a 1–2 min inter-trial interval [19].