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Research report

Early onset of forced impaired forelimb use causes recovery of forelimb skilled motor function but no effect on gross sensory-motor function after capsular hemorrhage in rats

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ABSTRACT

Intensive use of the impaired forelimb promotes behavioral recovery and induces plastic changes of the central nervous system after stroke. However, the optimal onset of intensive use treatment after stroke is controversial. In this study, we investigated whether early forced impaired limb use (FLU) initiated 24 h after intracerebral hemorrhage (ICH) of the internal capsule affected behavioral recovery and histological damage. Rats were subjected to ICH via low-dose collagenase infusion or sham stroke. One day after surgery, the ipsilateral forelimbs of half of the ICH and sham rats were casted for a week to induce the use of their contralateral forelimbs. Behavioral assessments were performed on days 10–12 and 26–28 after the surgery and followed by histological assessments. Improvements in skilled reaching and coordinated stepping function were found in the FLU-treated group in comparison with the untreated group after ICH. Additionally, FLU-treated ICH animals showed more normal and precise reaching and stepping movements as compared with ICH control animals. In contrast, FLU did not have a significant impact on gross sensory-motor functions such as the motor deficit score, contact placing response and spontaneous usage of the impaired paw. The volume of tissue lost and the number of spared corticospinal neurons in lesioned motor cortex were not affected by early FLU after ICH. These findings demonstrate the efficacy of early focused use of an impaired limb after internal capsule hemorrhage.

1. Introduction

Upper limb hemiparesis often occurs after ischemic or hemorrhagic stroke. Unilateral upper extremity impairment substantially disturbs patients' ability to complete activities of daily living [1]. Forced use of an impaired upper limb, known as constraint-induced movement therapy (CIMT), is a promising method for improvement of upper limb function after stroke [2–4]. Intensive use of the affected limb is thought to cause functional recovery by overcoming learned nonuse of the affected limb and causing use-dependent reorganization of the central nervous system (CNS) [5]. Some laboratory and clinical trials have reported that intensive use of an impaired forelimb or CIMT promoted functional recovery of the impaired limb [6,7]. However, it is not clear what the optimal conditions for efficacy of intensive use therapy are.

The onset of forced-use treatment after stroke is an influential factor that remains unclear. Generally, early rehabilitative training is assumed to have a beneficial impact on functional recovery because molecular events associated with recovery are initiated early after stroke [8]. Although there are wide variations among factors, many endogenous plasticity-related genes are upregulated within a few weeks following stroke [9,10]. In fact, immediate forced use of the impaired forelimb after unilateral corticospinal tract (CST) transection caused morphological alterations of intact CST axons and promoted functional recovery [11]. However, other reports have indicated that early intensive use training might have a detrimental effect. Previous rodent studies demonstrated that immediate exclusive use of the impaired limb after cortical injury attenuated functional recovery and exacerbated neural injury [12,13]. These conflicting reports indicate that the ideal onset of intensive use therapy remains controversial.

Additionally, most studies on forced-use therapy have focused on cerebral ischemia or other cortical injury models (e.g.,

Abbreviations: FLU, forced impaired limb use; IC, internal capsule; ICH, intracerebral hemorrhage; CIMT, constraint-induced movement therapy; CNS, central nervous system; CST, corticospinal tract; MDS, motor deficit score; FG, fluorogold.

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electrolytic lesions) [7,12,13]. In contrast, few studies have addressed the effects of CIMT after intracerebral hemorrhage (ICH). DeBow et al. demonstrated that CIMT started one week after ICH promoted functional recovery and reduced the volume of tissue loss [14]. However, the effects of CIMT begun immediately after ICH have not been investigated. ICH is a common type of stroke with different pathophysiology than cerebral infarcts and cortical lesions [15]. Previous studies demonstrated that ICH affected multiple factors involved in post-stroke recovery, such as trophic factor expression, microglial activation and neurogenesis [16,17]. It is possible that immediate forced use of the impaired limb may have different effects in ICH models than in ischemia and cortical lesion models. In addition, we showed that early application of treadmill running improved motor function and altered dendritic branching in ICH rats [18].

In this study, we investigated the effect of forced impaired limb use (FLU) started one day after ICH of the internal capsule (IC) on behavioral and histological recovery markers. We conducted several behavioral tests to characterize the effect of FLU on the motor function, assessed lesion volume and the volume of spared CST running through the IC.

2. Methods

2.1. Animals and experimental design

Adult male Wistar rats (250–300 g) were used. Animals were housed in groups of three to four animals in standard cages with a 12 h light/dark cycle and received food and water ad libitum except for the period of food deprivation. All procedures were in accordance with the animal care guidelines of Nagoya University. All efforts were made to minimize suffering and the number of animals used.

Animals were randomly divided into four experimental groups: (1) ICH-FLU: ICH with forced use of their impaired forelimb ($n = 8$), (2) ICH: ICH without any treatment ($n = 9$), (3) sham-FLU: no lesion with forced use of their dominant paw ($n = 6$), and (4) sham ($n = 6$).

2.2. Internal capsule hemorrhage

IC hemorrhage was generated according to methods described previously [19]. Briefly, rats were deeply anesthetized using sodium pentobarbital (45 mg/kg, intraperitoneally) and placed in a stereotaxic frame. After shaving and sanitizing the scalp, a midline scalp incision was made and a small hole drilled contralateral to the dominant paw at 3.8 mm lateral to midline, 1.8 mm caudal to bregma. The dominant paw of each rat was determined during reaching training (see Section 2.4.4). Using a glass micropipette (tip diameter, 50–60 μm), type IV collagenase (1.4 μl, 15 U/ml; Sigma Chemical Co., St. Louis, MO, USA) dissolved in sterile saline was injected 5 mm below the brain surface over 7 min. The needle was left in place for 7 min and then slowly removed. Sham groups received the same volume of saline infusion. Rectal temperature was maintained at $37.0 \pm 0.5\,^{\circ}C$ during surgery. After injection, the wound was closed and rats warmed under a heating lamp until they awoke.

2.3. Forced limb use

Twenty-four hours after surgery, FLU group rats were fitted with one sleeve cast. The unimpaired (ICH-treated rats) or nondominant (sham-operated rats) forelimb was placed in a retracted position against the sternum while under anesthesia. Inhalation anesthesia was used (isoflurane: 3% for induction, 1.5% for maintenance) because of the rapid awakening that would minimize the behavioral suppressant effects of anesthesia. The limb and upper torso were wrapped in soft felt and plaster of Paris strips to immobilize them. In non-FLU-treated rats, the upper torso was wrapped in same way, but both forepaws were left free. The rats remained in the casts for seven days. The casts' conditions were checked several times daily during the FLU period and the casts were quickly replaced when damaged. FLU-treated rats were forced to rely on their impaired or dominant forelimb completely in all daily activities. Casts were gently removed eight days after ICH without anesthesia.

2.4. Behavioral training and testing

2.4.1. Motor deficit score

To evaluate gross motor dysfunction, motor deficit score (MDS) analysis was carried out as reported previously with some modification [19,20]. This score consisted of four tests, each designed to measure the degree of motor deficit on a five-point scale (graded from 0 for normal movement to 4 for most severe dysfunction): (1) spontaneous ipsilateral rotation, graded from 0 for no circling to 4 for continuous circling; (2) beam walking ability, graded 0 for a rat that readily traversed a 2.4-cm-wide and 80-cm-long beam to 4 for a rat that fell from the beam within 10 s; (3) contralateral hindlimb retraction, which measured the ability of a rat to replace the hindlimb after a lateral displacement of 2–3 cm, graded from 0 (immediate replacement) to 4 (complete absence of retraction); and (4) bilateral forepaw grasp, which measures the ability to hold onto a 2-mm-diameter steel rod, graded 0 for immediate grasping to 4 for inability to grasp. The scores were added and expressed as MDS (maximum possible score is 16). Assessment was carried out 1, 12 and 28 days after ICH. Rats with an MDS of 8 or below at the first post-ICH assessment were excluded from the study.

2.4.2. Cylinder test

Asymmetry of forelimb usage was evaluated by the cylinder test [21]. Rats were placed in a Plexiglas cylinder (20 cm in diameter, 35 cm in height) and videotaped for 5 min. The number of independent uses of either forelimb and the simultaneous use of both forelimbs during wall movements were counted. The percentage of affected limb use was calculated using the formula: ((contralateral forelimb contacts + 1/2 bilateral contacts)/total contacts)) × 100. Rats were tested 12 and 28 days following ICH. Rats that made fewer than eight independent wall touches were excluded from analysis to ensure that enough touches were recorded to allow reliable measurement.

2.4.3. Contact placing response

To assess the sensorimotor integration of the forelimb, sensory stimulation-evoked placing response was observed [22]. Briefly, rats were held in the air with the forelimb suspended and advanced to the table. Replace response on the table was counted when the dorsum of the paw touched the edge of the table. The task was repeated five times and the percentage of responses calculated 12 and 28 days after ICH.

2.4.4. Single pellet reaching test

The single pellet reaching test was performed to evaluate forelimb skilled reaching function [23]. Rats were food-restricted throughout the reaching task training and testing periods so that they neither gained nor lost weight. Rats were placed in a transparent Plexiglas chamber (45 cm in height, 13 cm in width, 40 cm in length) and trained to reach through a 1 cm slit to retrieve precision sucrose pellets (45 mg; Bioserv Inc., Frenchtown, NJ, USA).

An external shelf (3 cm in width) with two indentations was used to present food pellets and was attached with the front wall of the apparatus (4 cm from the bottom). Each indentation was positioned 1.5 cm from the inside wall of the apparatus and aligned to either the left or right edge of the opening. Pellets were placed on either indentation to train their dominant forelimb, which was determined during the first practice session. Pre-operative training (20 trials/day) was carried out for three weeks, and rats that failed to obtain at least 10 pellets per session with their preferred limb during the last three sessions were excluded.

The last three pre-training sessions were defined as baseline tests. Each testing session consisted of 20 trials and was carried out once per day. For quantitative analysis, the average number of pellets rats retrieved without dropping them was measured for three consecutive days. Reaching movement was also evaluated as described by Metz and Whishaw with some modification [24]. Briefly, rats were videotaped during the final testing session and three successful reaches were analyzed frame-by-frame (60 frames/sec). If rats could not retrieve more than three pellets, they were given the opportunity to do another 20 trials. Each reach was decomposed into eight movement components: (1) Aim, (2) Advance, (3) Digits open, (4) Pronation, (5) Grasp, (6) Supination I, (7) Supination II and (8) Release. Each of the components was scored as 1 (normal movement), 0.5 (the movement present but abnormal) or 0 (movement absent). Scores from three reaches were averaged as the reaching score. The reaching score indicates the degree of abnormality for each reaching component. Following completion of the training period, the rats were allowed to access food ad libitum. Post-lesion testing including retrieved pellet number and reaching movement quality was conducted again 10–12 and 26–28 days after surgery.

2.4.5. Horizontal ladder test

The testing apparatus was a 1-m-long ladder with rungs spaced irregularly between 1 and 3 cm [25]. Rats were accustomed to the testing apparatus prior to surgery until they could cross a horizontal ladder at a constant speed. To encourage rats to cross the ladder, their home cage was set at the opposite side.

For behavioral testing, rats were videotaped crossing the ladder three times and each step was analyzed frame-by-frame (60 frames per second) while traversing the middle 0.6 m of the ladder. We calculated the percentage of fall steps (rungs slipped off of and missed) and correct steps (the middle of the palm of the forelimb was placed on the rung and all four digits were placed in front of the rung). Testing was performed on postoperative days 12 and 28.

2.5. Retrograde labeling

To label CST spared after ICH, retrograde tracer fluorogold (FG, 4%, w/v in saline, pH 7.4; Biotium, Hayward, CA, USA) was used 30 days after ICH. Rats were fixed on a stereotaxic frame under deep anesthesia. After C6–C8 laminectomy, FG was injected into the spinal cord using a Hamilton microsyringe with pulled glass tip. Each rat had four injections of 150 nl of FG spaced at 0.5 mm along the spinal cord. The injection