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BRAIN RESEARCH

Research Report

Forced exercise does not improve recovery after hemorrhagic stroke in rats

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ABSTRACT

Exercise can improve recovery following ischemia and intracerebral hemorrhage (ICH) in rodents. We tested whether forced exercise (EX; running wheel) prior to and/or following ICH in rats would reduce lesion volume and improve functional outcome (walking, skilled reaching, spontaneous paw usage) at 7 weeks post-ICH. A striatal hemorrhage was produced by infusing collagenase. First, we compared animals that received EX (2 weeks; 1 h/day) ending two days prior to ICH and/or starting two weeks following ICH. EX did not improve functional recovery or affect lesion size. Doubling the amount of EX given per day (two 1-h sessions) both prior to and following ICH did not alter lesion volume, but worsened recovery. We then determined if EX (1 h/day) prior to and following ICH would affect outcome after a somewhat milder insult. There were no differences between the groups in lesion volume or recovery. Finally, we used a hemoglobin assay at 12 h following ICH to determine if pre-stroke EX (2 weeks; 1 h/day) aggravated bleeding. It did not. These observations suggest that EX does not improve outcome when given prior to and/or when delayed following ICH. Effective rehabilitation for ICH will likely require more complex interventions than forced running.

1. Introduction

Stroke is one of the leading causes of death and disability in North America. Cerebral bleeding, including intracerebral hemorrhage (ICH), accounts for approximately 15% of all strokes and frequently causes severe disability or death (Mayo et al., 1982). Most experimental and clinical stroke studies focus on finding effective hemostatic or cytoprotective (neuroprotective) therapies. Furthermore, most studies target cerebral ischemia and not hemorrhagic stroke. Given the differences in pathophysiology (Lipton et al., 1999; Xi et al., 2006), such as the nature of cell death and the extent and location of injury, it is important to test therapies in ICH models as it is possible that treatments that work in ischemia may fail in ICH. This is not only an issue with cytoprotectants, but rehabilitation therapies may also differ in efficacy between ischemic and hemorrhagic events.

Various rehabilitation interventions promote recovery after ischemic stroke in rats (e.g., environmental enrichment). Interestingly, even simple exercise (EX) treatments such as forced running promote recovery after ischemic stroke, and they also reduce cell death in some situations. For example, Ding and colleagues (2004) found that forced

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EX prior to temporary middle cerebral artery occlusion (MCAO) reduced infarct volume and promoted neurological recovery, which was associated with increased expression of brain derived neurotrophic factor (BDNF) and nerve growth factor. In another study, pre-stroke forced EX reduced edema and lesion volume measured at 24 h after temporary MCAO (Wang et al., 2001). Reductions in lesion volume and improved behavioral recovery also occur when EX is started following ischemia. For instance, forced EX initiated within 24 h of temporary MCAO reduced functional deficits and lesion volume (Yang et al., 2003).

Rehabilitation also benefits rats suffering an ICH. DeBow and colleagues (2003) showed that constraint-induced movement therapy (CIMT) initiated one week after ICH improved behavioral recovery. A reduction in the total volume of tissue lost also occurred. The CIMT therapy, which lasted 7 days, included a combination of ipsilateral limb restraint (8 h/day) combined with 1 h/day of EX (e.g., skilled reaching training, walking). The modest EX treatment alone did not improve outcome. However, others have shown that greater amounts of forced EX (running) starting 1 day following ICH reduces lesion volume, caspase-3 expression, and the number of degenerating cells (Lee et al., 2003; Lee et al., 2005). This raises the possibility that greater amounts of EX may improve recovery and reduce injury when given after a more clinically realistic treatment delay (e.g., 2 weeks).

In this study, we examined the effects of forced EX on outcome following ICH in rats. The collagenase model of ICH, developed by Rosenberg and colleagues (1993), was used because it results in consistent hemorrhaging within the striatum and well-characterized behavioral deficits (Maclellan et al., 2005a). Forced EX, via motorized running wheels, was used because of its demonstrated efficacy in ischemia models and because an exact amount of EX can be easily administered. Effective pre-clinical testing requires a comprehensive assessment of long-term recovery and not just use of short-term endpoints (Corbett and Nurse, 1998). Thus, we used a 7-week survival time and gauged skilled reaching, spontaneous paw usage, and walking ability by the staircase, forelimb asymmetry, and horizontal ladder tests, respectively, which are all sensitive to striatal ICH (Hua et al., 2002; Maclellan et al., 2005a). In our first experiment, we examined the effects of EX given before and/or after ICH. We hypothesized that EX either before or following ICH would be beneficial and expected the combination to be superior. The second experiment doubled the amount of daily EX given both before and following ICH. We hypothesized that this EX regimen would lead to greater benefit. In Experiment 3, we reduced the lesion size to determine the effects of EX given both before and after ICH as in Experiment 1. We anticipated somewhat greater effects in treating this smaller lesion than that found in Experiment 1. Lastly, in Experiment 4 we quantified hemorrhage volume at 12 h after ICH to determine if forced EX prior to ICH aggravated bleeding. Given the known angiogenic effects of EX (Black et al., 1990; Kleim et al., 2002; Swain et al., 2003), we predicted that pre-ICH EX treatment might aggravate bleeding and thereby counteract beneficial effects of EX therapy.

2. Results

2.1. *Weight data*

In all experiments, body weight was similar among all groups at the beginning of the experiment, on the first and last day of PRE and POST EX treatments, surgery, post-surgery, and euthanasia ($p \geq 0.066$).

2.2. *Experiment 1*

No animals in the POST-1 group died, whereas 1 in the CONT-1 group, 4 animals in the PRE&POST-1 group, and 1 animal from the PRE-1 group died following surgery ($p = 0.095$). The cause of death was presumed to be due to insult severity; however, this was not verified.

The lesion volumes at 7 weeks after ICH are given in Fig. 1A. Injection of collagenase caused significant tissue loss in the striatum as well as damage to thalamus, globus pallidus, and the corpus callosum (Fig. 1D). The main effects (PRE: $p = 0.412$; POST: $p = 0.212$) and interaction ($p = 0.419$) were not significant.

All groups performed similarly during staircase training (data not shown). There was a significant Day effect ($p = 0.001$; Fig. 2A) because all groups improved over time (contralateral limb reaching success). However, the PRE ($p = 0.820$) and POST ($p = 0.440$) main effects and the interaction ($p = 0.959$) were not significant. Likewise, the main effects (PRE: $p = 0.586$; POST: $p = 0.391$) and interaction ($p = 0.136$) were not significant for the contralateral forelimb slip rate in the ladder test (Fig. 3A). Analysis of contralateral limb use in the asymmetry test revealed a significant POST main effect ($p = 0.039$; Fig. 4A) and a significant PRE × POST interaction ($p = 0.033$), but the PRE factor was not significant ($p = 0.075$). However, Scheffé post hoc tests revealed no significant differences between any of the groups ($p \geq 0.065$) in a one-factor ANOVA. The composite score revealed that neither the main effects (PRE: $p = 0.811$; POST: $p = 0.178$) nor interaction ($p = 0.868$) were significant. The CONT-1, PRE-1, POST-1, and PRE&POST-1 groups had mean composite scores (lower reflects better performance) of 35.1 ± 12.7, 34.9 ± 11.7, 31.6 ± 12.6, and 30.5 ± 8.6, respectively.

2.3. *Experiment 2*

Two animals in the PRE&POST-2 group died after ICH whereas no animal died in the CONT-2 group ($p = 0.176$). Mortality was assumed to be due to the ICH.

Histological data for one animal in the CONT-2 group was lost before analysis. The one-factor ANOVA revealed no significant difference in lesion size between the PRE&POST-2 and CONT-2 groups ($p = 0.465$; Fig. 1B).

There was no significant difference in contralateral reaching ability between the two groups ($p = 0.052$) and the Day effect was not significant ($p = 0.090$; Fig. 2B). As shown in Fig. 3B, the PRE&POST-2 group made significantly more foot slips with their contralateral forelimb than the CONT-2 group ($p = 0.032$). Analysis of limb use asymmetry revealed no significant difference between the groups ($p = 0.395$; Fig. 4B).