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Research report

Early rehabilitation aggravates brain damage after stroke via enhanced activation of nicotinamide adenine dinucleotide phosphate oxidase (NOX)

Jiamei Shen [a,b], Mitchell Huber [b], Ethan Y. Zhao [b], Changya Peng [b], Fengwu Li [a], Xiaorong Li [a], Xiaokun Geng [a,b,*], Yuchuan Ding [a,b,**]

[a] China-America Institute of Neuroscience, Beijing Luhe Hospital, Capital Medical University, China
[b] Department of Neurosurgery, Wayne State University School of Medicine, Detroit, MI, USA

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ABSTRACT

Introduction: Although physical exercise has emerged as a potential therapeutic modality for functional deficits following ischemic stroke, the extent of this effect appears to be contingent upon the time of exercise initiation. In the present study, we assessed how exercise timing affected brain damage through hyperglycolysis-associated NADPH oxidase (NOX) activation.

Methods: Using an intraluminal filament, adult male Sprague-Dawley rats were subjected to middle cerebral artery occlusion (MCAO) for 2 h and assigned to one non-exercise and three exercise groups. Exercise on Rota-rod was initiated for 30 min at 6 h (considered very early), at 24 h (early), and at day 3 (relatively late) after reperfusion. Lactate production was measured 30 min after exercise completion, and NOX activity and protein expression of NOX subunits ($p47^{phox}$, $gp91^{phox}$, $p22^{phox}$ and $p67^{phox}$) and glucose transporter 1 and 3 (Glut-1 and -3) were measured at 3 and 24 h after exercise. Apoptotic cell death was determined at 24 h after exercise.

Results: Lactate production and Glut-1 and Glut-3 expression were increased after very early exercise (6 h), but not after late exercise (3 days), suggesting hyperglycolysis. NOX activity was increased with the initiation of exercise at 6 h ($P < 0.05$), but not 24 h or 3 days, following stroke. Early (6 and 24 h), but not late (3 days), post-stroke exercise was associated with increased ($P < 0.05$) expression of the NOX protein subunit $p47^{phox}$, $gp91^{phox}$and $p67^{phox}$. This may have led to the enhanced apoptosis observed after early exercise in ischemic rats.

Conclusion: Hyperglycolysis and NOX activation was associated with an elevation in apoptotic cell death after very early exercise, and the detrimental effect of exercise on stroke recovery began to decrease when exercise was initiated 24 h after reperfusion.

1. Introduction

Ischemic stroke leads to profound neurological deficits and lasting physical disability. To date, stroke remains the leading cause of major disability worldwide. The use of exercise to reduce physical disability after stroke has become an emerging field in neurotherapeutics (Ding et al., 2003, 2006; Lee et al., 2009). Accumulating evidence indicates that physical exercise improves functional outcome and induces neuronal plasticity, and that there may be a window of opportunity following injury where exercise-dependent structural changes exert their greatest effect. As such, early exercise rehabilitation after stroke has received significant attention. In experimental animal stroke models, some studies have suggested a beneficial effect of exercise if initiated as early as 24 h after onset of ischemic (Matsuda et al., 2011) or hemorrhagic stroke (Park et al., 2010). In contrast, other studies have reported a negative impact on functional recovery, lesion volume, and the expression of proteins involved in neuroregeneration after early initiation of physical activity (Humm et al., 1998; Risedal et al., 1999; Steultjens and Jellema, 2014). Furthermore, increased injury was detected in the forelimb area of the sensorimotor cortex in rats forced to overuse the impaired forelimb for 7 (Humm et al., 1998) or 15 days following ischemic stroke (Kozlowski et al., 1996). Thus, a review of the literature regarding post-stroke exercise

** Correspondence to: Department of Neurological Surgery, Wayne State University School of Medicine, 550 E Canfield, Detroit, MI 48201, USA.
* Correspondence to: Department of Neurology, Beijing Luhe Hospital, Capital Medical University, No. 82 Xinhua South Road, Tongzhou District, Beijing 101149, China.
E-mail addresses: xgeng@med.wayne.edu (X. Geng), yding@med.wayne.edu (Y. Ding).

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presents a conflicting picture of the effect of early exercise after stroke (Hotting and Roder, 2013), suggesting that very early activity, in fact, exacerbates brain injury (Hsu and Jones, 2005). However, how exercise timing affects brain injury remains unclear.

Ischemia is well known to induce cerebral hyperglycolysis (Schurr, 2002), and reports indicate that this hyperglycolysis may exacerbate brain damage after stroke (Bergsneider et al., 1997). Following ischemia/reperfusion, the brain substantially increases glycolytic rate, as shown by increased expression of glucose transporter 1 and 3 (Glut-1 and -3) (Dornbos et al., 2013), and lactic acidosis (Schurr, 2002). The excess glucose is consequently routed into anaerobic respiration, and the hexose monophosphate pathway where it activates nicotinamide adenine dinucleotide phosphate oxidase (NOX), as shown by increased NOX activity and expression of NOX subunits ($p47^{phox}$, $p67^{phox}$, and $gp91^{phox}$), leading to cell death (Tang et al., 2012a, 2012b).

The aim of the present study was to determine if post-stroke hyperglycolysis is enhanced by early exercise compared to late exercise and whether an increase in glucose metabolism is associated with elevations in NOX activation and NOX subunit expression, leading to attenuated apoptotic cell death in rats subjected to a 2-hour middle cerebral artery occlusion (MCAO). Previous laboratory studies using rat models have implemented exercise as early as 24–48 h post–ischemia (Lee et al., 2009; Matsuda et al., 2011) and chosen 4 or 5 days for initiating relatively late exercise (Ding et al., 2003; Ding et al., 2004; Tamakoshi et al., 2014). In the present study, we proposed a spectrum of times (6 h, 24 h, and 3 days after the onset of reperfusion) for exercise initiation to determine optimal time of exercise implementation.

2. Results

2.1. Physiological parameters

No significant differences in blood PO_2, PCO_2 or pH were found in ischemic rats during surgery before either receiving or not receiving exercise. Body temperature remained at 37 °C throughout the course of surgery.

2.2. Apoptotic cell death

Stroke increased apoptotic cell death measured in all stroke without exercise groups after reperfusion (7.7 ± 0.3, 8.5 ± 0.8, 8.0 ± 0.6 fold; $P < 0.05$) compared to the sham control, which was assigned a value of 1 to serve as a reference (Table 1). With very early exercise initiated at 6 h post-reperfusion, apoptotic cell death was further elevated (11.7 ± 0.7 fold; $P < 0.05$) compared to the non-exercise stroke group at the same time point. However, with exercise initiated at 24 h after reperfusion, apoptotic cell death was not significant compared to the corresponding non-exercise stroke group. With late exercise (3 d) post-reperfusion, apoptotic cell death was also not significantly different compared to that in non-exercise.

2.3. Cerebral lactate levels

While sham lactate levels were 7.5 ± 0.3 nmol/mg, stroke significantly ($P < 0.05$) increased lactate levels in all 3 no-exercise stroke groups (Fig. 1A). Exercise initiated at 6 h post-reperfusion significantly ($P < 0.05$) elevated cerebral lactate levels to 10.0 ± 0.2 nmol/mg compared to 9.2 ± 0.4 nmol/mg in the non-exercise groups. However, in groups where exercise was initiated 24 h after reperfusion, lactate levels were significantly ($P < 0.05$) decreased (7.8 ± 0.4 nmol/mg) compared to non-exercise at the same time point (9.9 ± 0.7 nmol/mg). Lactate levels were also significantly ($P < 0.05$) decreased after late exercise (3 d) (7.4 ± 0.4 nmol/mg) compared to non-exercise (8.8 ± 0.7 nmol/mg) (Fig. 1B). Thus, very early exercise (6 h) led to a significant increase in lactate levels, while exercise at 24 h and 3d after reperfusion did not.

2.4. Protein expressions of Glut-1

Glut-1 expression was increased ($P < 0.05$) at 3 h (1.7 ± 0.1 fold) and 24 h (2.0 ± 0.2 fold) after completion of early exercise that was initiated 6 h after reperfusion compared to no-exercise control (1.4 ± 0.2 and 1.5 ± 0.2 fold, respectively) (Fig. 2A). Similarly, exercise initiated 24 h after reperfusion led to significant increases in Glut-1 expression at 3 h (2.4 ± 0.3 fold, $P < 0.01$) and 24 h (2.0 ± 0.2 fold, $P < 0.05$) after exercise compared to non-exercise at 3 h (1.5 ± 0.2 fold) and 24 h (1.4 ± 0.2 fold) (Fig. 2B). However, exercise initiated 3 d after reperfusion did not result in a significant increase in Glut-1 expression at 3 h (1.1 ± 0.2 fold) and 24 h (1.2 ± 0.1 fold) after exercise completion compared to non-exercise at the same time points (1.7 ± 0.2 at 3 h, 1.4 ± 0.3 at 24 h) (Fig. 2C).

2.5. Protein expressions of Glut-3

Stroke increased Glut-3 production at all time points measured when compared to sham control. When exercise was initiated 6 h after reperfusion, Glut-3 expression was further increased at 3 h (1.5 ± 0.1, $P < 0.05$) and 24 h (1.8 ± 0.2, $P < 0.05$) after exercise termination compared to non-exercise at the same time points (1.3 ± 0.0 at 3 h and 1.3 ± 0.1 at 24 h) (Fig. 3A). Exercise initiated 24 h after reperfusion led to similar increases ($P < 0.05$) (2.0 ± 0.2 at 3 h after exercise compared to 1.6 ± 0.1 for control; 1.8 ± 0.2 at 24 h after completion compared to 1.30 ± 0.2 for control) (Fig. 3B). In ischemic rats with exercise started 3 d after reperfusion, Glut-3 expressions at both 3 h (1.4 ± 0.1, $P < 0.05$) and 24 h (1.1 ± 0.1, $P < 0.01$) were lower than that of non-exercise at the same time points (1.7 ± 0.1 at 3 h and 1.6 ± 0.1 at 24 h) (Fig. 3C).

2.6. NOX activity

Stroke without exercise increased NOX acidity at every time point evaluated. Following very early (6 h) post-stroke exercise (Fig. 4A), NOX activity was further elevated at 3 h (1.8 ± 0.2 fold; $P < 0.01$) and 24 h (1.7 ± 0.3 fold; $P < 0.05$) compared to non-exercise at the same time point of 3 h (1.3 ± 0.2 fold) and 24 h

Table 1
Apoptotic Cell death.

Timing	Sham (No Stroke, No Exercise)	Non-exercise (Control)			Exercise		
		6 h	24 h	3 days	6 h	24 h	3 days
Cell death (Mean fold relative to control ± SE)	1.0 ± 0.7	$7.7 \pm 0.3^{**}$	$8.5 \pm 0.8^{**}$	$8.0 \pm 0.6^{**}$	$11.7 \pm 0.7^{\#}$	6.2 ± 0.5	5.0 ± 0.5

** $P < 0.01$ compared to sham (no stroke, no exercise) control.
# $P < 0.05$ compared to control (no exercise) group.