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Long-term effects of melatonin after intracerebral hemorrhage in rats

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Summary

Free radical scavengers have been shown to improve short-term outcome after intracerebral hemorrhage (ICH). The purpose of this study was to evaluate whether melatonin (a potent free radical scavenger and an indirect antioxidant) can improve short- and/or long-term neurological function after ICH, which was induced by collagenase injection into the striatum of adult rats. Melatonin (15 mg/kg) was administered by intraperitoneal injection at 1, 24, 48, and 72 h. Neurological and behavioral testing was performed at several time points from 1 day to 8 weeks post-ICH. Neurological and behavioral deficits were observed in ICH rats at all time points, but the melatonin treatment regimen did not improve performance or level of brain injury.

Keywords: Hemorrhage; collagenase; learning; memory; motor; melatonin.

Introduction

Intracerebral hemorrhage (ICH) represents at least 10–15% of all strokes in the Western population [13] and is often fatal. The collagenase-induced ICH rat model was originally described by Rosenberg *et al.* [14] and has been extensively used to evaluate the injury mechanisms and potential treatment regimens after ICH. However, the long-term effects after ICH in this model are not well understood. There are several published studies that have tested the behavioral effects of various therapeutic strategies following collagenase-induced ICH within the basal ganglia [1, 3, 5, 9, 10, 12], with most using motor tasks as the sole behavioral outcome measure to assess the efficacy of treatment strategies. However, cognitive deficits following ICH in humans are among the most prominent and troubling [7, 11, 15], with increasing evidence suggesting that the basal ganglia play a role in cognitive skills such as learning and memory. Our study examined whether melatonin reduces infarct size and/or neurological and cognitive deficits after collagenase-induced ICH in rats.

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Materials and methods

Intracerebral hemorrhage

Adult male Sprague-Dawley rats were divided into sham-operated controls and ICH groups and anesthetized. Injecting collagenase (VII-S; Sigma-Aldrich, St. Louis, MO) into the basal ganglia with a microinfusion pump (Harvard Apparatus, Holliston, MA) induced ICH, as previously described [16]. The needle was left in place for an additional 10 min after injection to prevent collagenase leakage. Sham surgery was performed with needle insertion alone.

Melatonin treatment

After surgery, ICH rats were divided into 2 groups (ICH + vehicle; ICH + 15 mg/kg melatonin). Intraperitoneal injections were administered at 1, 24, 48, and 72 h post-ICH. Sham rats were given vehicle injections.

Behavioral testing

A number of behavioral tests were administered through 8 weeks post-ICH, including a battery of neurological tests [6], accelerating Rotarod, open field, and water maze. This battery of tests allowed for repeated testing of a number of behavioral domains, including neurological reflexes, sensorimotor coordination and balance, motor learning, general activity levels, cued learning, spatial learning, short- and long-term memory, swim speed, and turn bias.

Histology

Brains were removed at 10 weeks post-ICH, sliced into 10 μm thick sections, stained with cresyl violet Nissl stain, and infarct size was evaluated (Table 1).

Table 1. *Infarct size (percent of contralateral hemisphere)*

Treatment group	Infarct size
Sham + Vehicle	0
ICH + Vehicle	10 ± 3
ICH + 15 mg/kg melatonin	12 ± 4

Results

Neurobehavioral testing

Although behavioral deficits, including cued and spatial learning, spatial memory, and sensorimotor coordination were observed in ICH rats throughout the 8-week test period, the melatonin treatment regimen produced no significant change in behavior.

Histology

Treatment with melatonin did not produce any significant changes in the area of injury produced by collagenase-induced ICH.

Discussion

The collagenase-induced ICH model produces lesions in the dorsolateral and middle regions of the striatum [8], which are important areas for the control of skilled motor function [17]. This model often produces a functional impairment of fine motor control of the distal forelimb and paw as revealed by the staircase test [3]. Neurological deficits in this model are most severe from 24–72 h after ICH induction and are typically attenuated by 1 month [4, 5, 14]. However, long-term neurological deficits have been shown when evaluating rotational bias in response to amphetamine and contralateral stepping [2]. In the current study, this model of ICH produced detectable cognitive and motor deficits in rats over an 8 week time period. Along with histological analysis of infarct volume, this characterization provides a suitable baseline for the analysis of therapeutic intervention strategies. However, melatonin, at the dose and frequency tested, did not improve infarct size or neurological function of rats after ICH induction. It is possible the melatonin regimen was ineffective at reducing the oxidative damage produced by ICH, or that processes other than oxidative stress were responsible for the behavioral deficits and brain damage. It remains to be determined whether other antioxidant agents can prevent the long-term behavioral deficits that were characterized in this model.

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