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Research report

Enriched rehabilitation promotes motor recovery in rats exposed to neonatal hypoxia-ischemia

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HIGHLIGHTS

- A combination therapy of cyclosporine A and enriched rehabilitation is evaluated.
- Enriched rehabilitation promotes motor recovery.
- Sensitive behavioural tests can detect early impairments in hypoxic-ischemic rats.
- CsA given 2 weeks after HI is not therapeutically efficacious.

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ABSTRACT

Despite continuous improvement in neonatology there is no clinically effective treatment for perinatal hypoxia ischemia (HI). Therefore, development of a new therapeutic intervention to minimize the resulting neurological consequences is urgently needed. The immature brain is highly responsive to environmental stimuli, such as environmental enrichment but a more effective paradigm is enriched rehabilitation (ER), which combines environmental enrichment with daily reach training. Another neurorestorative strategy to promote tissue repair and functional recovery is cyclosporine A (CsA). However, potential benefits of CsA after neonatal HI have yet to be investigated. The aim of this study was to investigate the effects of a combinational therapy of CsA and ER in attempts to promote cognitive and motor recovery in a rat model of perinatal hypoxic-ischemic injury. Seven-day old rats were submitted to the HI procedure and divided into 4 groups: CsA + Rehabilitation; CsA + NoRehabilitation; Vehicle + Rehabilitation; Vehicle + NoRehabilitation. Behavioural parameters were evaluated pre (experiment 1) and post 4 weeks of combinational therapy (experiment 2). Results of experiment 1 demonstrated reduced open field activity of HI animals and increased foot faults relative to shams in the ladder rung walking test. In experiment 2, we showed that ER facilitated acquisition of a staircase skilled-reaching task, increased number of zone crosses in open-field exploration and enhanced coordinated limb use during locomotion on the ladder rung task. There were no evident deficits in novel object recognition testing. Delayed administration of CsA, had no effect on functional recovery after neonatal HI. There was a significant reduction of cortical and hemispherical volume and hippocampal area, ipsilateral to arterial occlusion in HI animals; combinational therapy had no effect on these morphological measurements. In conclusion, the present study demonstrated that ER, but not CsA was the main contributor to enhanced recovery of motor ability after neonatal HI.

Abbreviations: HI, hypoxia-ischemia; ER, enriched rehabilitation; CsA, cyclosporine A; PND, postnatal day.

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1. Introduction

Perinatal hypoxia ischemia (HI) is one of the most common causes of mortality and morbidity in children [1,2]. Despite improvement in neonatal care there is no clinically effective treatment for this disorder. Therefore, development of a new therapeutic intervention to minimize the resulting neurological consequences of cerebral palsy, mental retardation and learning disabilities [3–5] is urgently needed.

The Rice-Vannucci model is widely used to study neonatal encephalic HI in rodents [6]. The main brain structures affected in this model are cerebral cortex, hippocampus, striatum and thalamus, mostly confined to the hemisphere ipsilateral to arterial occlusion [7,8]. As in humans, rats that experience cerebral HI have motor and cognitive deficits [9–14]. However, there is some inconsistency regarding motor impairments after HI lesion; some authors have found no impairment in forelimb use or motor coordination in adult rats exposed to neonatal HI [12,15,16].

Environmental enrichment has been used as a strategy to enhance neuroplasticity and to promote recovery of function following different types of brain injury such as stroke and HI [7,17–21]. Enriched housing provides sensory, cognitive and motor stimulation as well as social interaction by exposing groups of animals to a variety of objects such as ramps, toys, and other novel objects. Several studies have demonstrated that environmental enrichment can attenuate learning and memory deficits in HI rats [7,14,15,22]. However, motor impairments are not always rescued by exposure to an enriched environment. For example, some authors found no improvement on rotarod [15,19] and foot fault tests [23] thereby emphasizing the importance of conducting comprehensive test batteries. A more effective paradigm, especially for restoring upper limb function, is enriched rehabilitation (ER), which combines environmental enrichment with daily reach training [24]. Enriched rehabilitation improves forelimb and hindlimb motor function following both focal ischemic injury [24–26] as well as hemorrhagic stroke [27,28] in adult animals.

Another approach to enhance recovery is using drugs with pleiotropic actions such as Cyclosporine A (CsA) that has documented neurorestorative effects in adult animals with ischemic brain injury. This immunosuppressive drug enhances the activation of endogenous precursors to promote tissue repair that is correlated with functional recovery [29,30]. Additionally, CsA alters mitochondrial membrane permeability and transition pores so as to reduce oxidative damage [31,32] and recent data suggest that it reduces lipid peroxidation, apoptosis and neuroinflammation in a young rat model of closed head injury [33]. However, potential benefits of CsA after neonatal HI have yet to be investigated [34].

An emerging consensus is that interventions targeting single mechanisms are not successful in treating stroke and related neurological disorders. Instead, combination interventions targeting multiple mechanisms and thereby mimicking endogenous programs of neuroprotection and neural repair offer greater potential benefit [35]. Recently, we used this approach to show that ER combined with Erythropoietin (EPO) and epidermal growth factor (EGF) was more effective than the growth factors or ER alone in promoting behavioural recovery following forelimb motor cortex stroke in rats [36]. In the same vein, the present study was undertaken to examine effects of a combinational therapy consisting of CsA and ER in attempts to promote cognitive and motor recovery in a rat model of perinatal hypoxic-ischemia injury.

2. Materials and methods

2.1. Animals

Seven pregnant Sprague-Dawley rats were acquired from Charles River Laboratories (Montreal, Quebec, Canada) and housed on a reverse 12 h light/dark cycle until parturition with food and water freely available. At post-natal day 7 (PND) pups from the 7 dams were randomly divided into two experimental groups: HI (n = 17 females; n = 14 males) and sham (n = 9 females; n = 9 males). Subsequent behavioural testing was done during the dark phase. All procedures were in accordance with guidelines set by the Canadian Council on Animal Care and the University of Ottawa Animal Care Committee.

2.2. Surgical procedures

At PND 7, rat pups were anesthetized with isoflurane, had their left common carotid artery exposed, isolated from the nerve and vein and ligated using 4-0 surgical silk. After a 2.5 h delay, pups were placed in a hypoxic chamber for 90 min with O_2 levels and temperature maintained at 8% and 37 °C respectively [5,6]. Upon conclusion of this hypoxic episode, pups were returned to their home cage. Sham-operated animals were submitted to manipulation, anesthesia and neck incision, but did not receive arterial occlusion or exposure to the hypoxic environment.

2.3. Cyclosporine A (CsA) administration

Our initial plan was to begin CsA administration (15.0 mg/kg, i.p.) in the first 5–14 days after HI as we have done previously with ER and drug therapies in adult animals [25,36]. Unfortunately, we encountered an extremely high mortality rate (~50%) in neonatal rats which was due to toxicity of the CsA vehicle Cremophor EL [37] since equal numbers of vehicle and CsA treated pups died. Consequently, we delayed CsA until weaning (PND 21), when all HI pups were implanted subcutaneously on the flank with osmotic minipumps (Alzet, Cupertino, USA) delivering CsA (420 mg/mL; BioShop, Burlington, Canada) or vehicle (Cremophor EL; ethanol:cremophor—65:35). Rats were anesthetized during osmotic minipump implantation with 1.5%-2% isoflurane. Pumps had a total fill volume of 100 μl and pumped at a rate of 0.14 μl/h [2]. The vehicle and/or CsA solutions at this later developmental time point resulted in no deaths or detectable morbidity. Minipumps were kept in place until the end of experiments.

2.4. Enriched rehabilitation

At PND 21, after osmotic pump implantation, pups were weaned from their mothers and separated by sex (Fig. 1A). Animals in groups receiving ER were housed in large enrichment cages (groups of four to five, Fig. 1B) while those in non-rehabilitation groups were pair housed in standard cages. Experimental groups were defined with respect to ER condition and drug delivery. Rats were randomly divided into four experimental groups: (CsA or vehicle): CsA + Rehabilitation (n = 4 females; n = 4 males); CsA + NoRehabilitation (n = 5 females; n = 2 males); Vehicle + Rehabilitation (n = 4 females; n = 5 males); Vehicle + NoRehabilitation (n = 4 females; n = 3 males).

Enrichment cages contained objects of varied shapes and texture for exploration (i.e., shelves, plastic tubing, ladders, ramp) that were changed on a weekly basis. In addition to being housed in enriched environments, enriched groups were exposed to rehabilitative reach training 4 h/day, 6 days/week for 4 weeks (PND 21 until PND 48). Rehabilitation consisted of reaching for a food reward in a Plexiglas© chamber using only the impaired limb (right). The reaching apparatus was filled with 8 g of sugar pellets (45 mg; Research Diets, New Brunswick, NJ) and was used to encourage (rather than force) coordinated use of affected forelimb. Procedures for rehabilitative reach training were modified from those described previously [25]. Animals were trained to reach through a 1.1-cm-wide vertical slot to obtain food pellets situated in a well