Int. J. Devl Neuroscience 55 (2016) 1–8

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Neuroprotective effects of hypothermia in inflammatory-sensitized hypoxic-ischemic encephalopathy

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ARTICLE INFO

Article history:
Received 14 July 2016
Received in revised form 5 September 2016
Accepted 6 September 2016
Available online 9 September 2016

Keywords:
Neonatal encephalopathy
Inflammation
Hypothermia
Neuroprotection

ABSTRACT

Background: Despite the recent introduction of hypothermia as a mandatory standard of care, the incidence of neonatal encephalopathy in full-term newborns and its devastating neuro-behavioral outcomes continues to be a major individual, familial and social issue. Neonatal encephalopathy is mainly due to the compounding and interacting effects of hypoxia-ischemia and inflammation resulting from placental and other perinatal infections. It is unclear why hypothermia is effective in alleviating neonatal encephalopathy in some, but not all, full-term newborns. However, newborns exposed to inflammatory-sensitized hypoxia-ischemia seem to have less therapeutic benefit from hypothermia than those exposed to hypoxia-ischemia alone.
Objectives: To clarify this uncertainty, we tested the efficacy of hypothermia in a double-hit model of neonatal encephalopathy induced by inflammatory-sensitized hypoxia-ischemia.
Methods: Using a rat preclinical model of endotoxin plus hypoxia-ischemia-induced neonatal encephalopathy of term newborns, we assessed the following in pups exposed (or not) to hypothermia: the extent of brain injuries and the expressions of molecules implicated in neural cell death, namely: pro-inflammatory cytokines, matrix metalloproteinase-9, antioxidant enzymes, as well as receptor-interacting protein-3.
Results: Hypothermia was neuroprotective on inflammatory-sensitized hypoxia-ischemia-induced penumbra, but not core, brain injuries. This beneficial effect was associated with a hypothermia-induced increase of antioxidant enzymes (superoxide dismutase-1, glutathione peroxidase-1), but was not associated with any variations of the other inflammatory mediators tested, namely: interleukin-1β, interleukin-1 receptor antagonist, tumor necrosis factor-α and matrix metalloproteinase-9.
Conclusion: Hypothermia is neuroprotective against inflammatory-sensitized hypoxia-ischemia possibly through a hypothermia-induced increase of antioxidant enzymes. This neuroprotective effect seems to be independent of the interleukin-1 system.

1. Introduction

Neonatal encephalopathy (NE) affects up to 0.8% of term newborns and is the second most common cause of childhood neurological disability (Azzopardi et al., 2014). Ongoing research surrounding neuroprotective strategies against NE is important as neuroprotective therapies on full-term newborns have resulted in conflicting findings in the literature (Azzopardi et al., 2014; Garfinkle et al., 2015). Treatments available against NE of full-term newborns consist of symptomatic care and hypothermia (HT),

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http://dx.doi.org/10.1016/j.ijdevneu.2016.09.002
0736-5748/

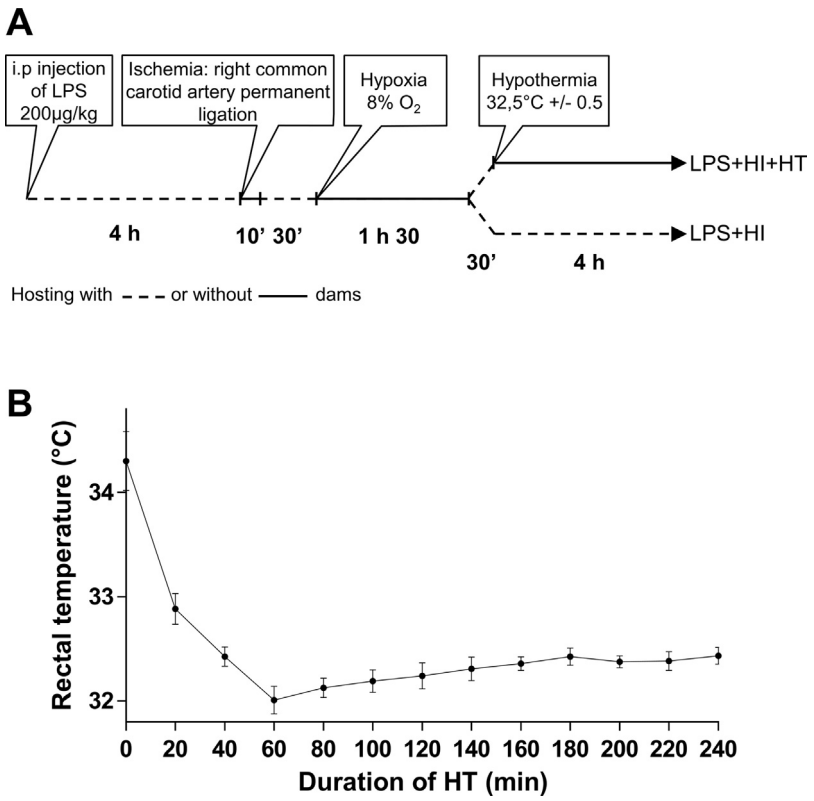

Fig. 1. Experimental design. Inflammation was induced in pups at postnatal day 12 (P12) with an i.p. injection of 200 μg/kg of LPS from *E. coli*; 4 h later, the right common carotid artery was ligated, and hypoxia was induced (8% O_2 for 1.5 h). Rat pups were subjected or not to HT (32.5 °C ± 0.5 °C for 4 h) (A). Rat pups reached the targeted temperature (32.5 °C ± 0.5 °C) 30 min ± 10 min after being isolated from the mother. Rectal temperature was measured every 20 min by using a calibrated temperature probe. The end of hypoxia was referred to as 0 min. Number (n) of rats: LPS + HI + HT (n = 12 from 6 litters) (B).
Abbreviations: h: hours; HI: hypoxia-ischemia; HT: hypothermia; i.p.: intraperitoneally; LPS: lipopolysaccharide from *E. coli*; min: minutes.

which leave 50% of the treated patients with major sequelae, such as cerebral palsy (CP) (Azzopardi et al., 2014). Data from the Canadian CP Registry showed that HT only prevents 4% of CP cases (Garfinkle et al., 2015). NE often arises due to the compounding effects of hypoxia-ischemia (HI) and inflammation resulting from placental and other perinatal infections (Fleiss et al., 2015; Girard et al., 2009b). It is unclear why HT is effective in alleviating NE in some, but not all, human term newborns. However, it has been reported that these newborns exposed to inflammatory-sensitized HI might have less of a therapeutic benefit from HT than those exposed to HI alone (Thoresen, 2015; Wintermark et al., 2010). Clinical and experimental studies showed that inflammatory and oxidative pathways implicating the interleukin-1 (IL-1) system, – namely the IL-1β/IL-1 receptor antagonist (IL-1Ra) ratio – tumor necrosis factor alpha (TNF)-α, matrix metalloproteinase-9 (MMP-9), and reactive oxygen species (ROS) play a critical role in brain injuries of term newborns resulting from HI or inflammatory-sensitized HI (Eliwan et al., 2015; Fleiss et al., 2015). Preclinical studies have established the neuroprotective effect of HT in the context of pure (without associated infection/inflammation) HI encephalopathy (HIE) in term-equivalent (postnatal day (P)10-13 pups) or late preterm-equivalent newborn pups (P7) (Patel et al., 2015, 2014; Vannucci et al., 1999). Few preclinical studies investigated the effect of HT when NE results from the combination of HI and infection/inflammation, a common pathophysiological scenario encountered in human term newborns with NE and subsequent CP (Fleiss et al., 2015; Savard et al., 2015). These findings led us to test, using a preclinical model (Savard et al., 2015), the neuroprotective efficacy of HT against NE due to inflammatory-sensitized HI at term.

2. Materials and methods

2.1. Rat model

Our pre-clinical model was designed as described (Savard et al., 2015, 2013). Briefly, Lewis dams (n = 10) were obtained from Charles River Laboratories (Saint-Constant, QC) between gestational day 13 (G13) and 15 (G15). At P12, pups received a single intraperitoneal (i.p.) injection of lipopolysaccharide (LPS: 200 μg/kg diluted in 50 μL of pyrogen-free saline) from *Escherichia coli* (*E. coli*; Sigma-Aldrich, ON). HI was induced 4 h after LPS administration by permanent ligation of the right common carotid artery followed by 8% O_2 exposure at 36 °C for 1.5 h (Brochu et al., 2011). Each session of surgery was performed in groups of 10 to 20 animals from 2 to 4 litters. In this model, the end of hypoxia is referred to as 0 h. In the LPS + HI + HT group, HT was induced 30 min after hypoxia. Pups on HT were kept on a hot plate in order to lower their temperature until 32.5 °C ± 0.5 °C (Fig. 1A). Pups reached the targeted temperature after 30 min ± 10 min. Rectal temperature was measured in all pups submitted to HT (every 20 min in each pup) by using a calibrated (0.1 °C deviation) temperature probe (RET-3-ISO, Physitemp Instruments, Clifton, NJ). HT was maintained in a reproducible manner for 4 h (Fig. 1B). In the LPS + HI group, pups stayed with the dam during the time their peers underwent HT; they maintained their temperature at 36.2 °C ± 0.4 °C (rectal temperature measured every hour in 6 sentinel pups used in our study). Among pups exposed to LPS + HI (n = 93), regardless of HT, the mortality rate was 8% during surgery, 45% during hypoxia, and 2% post-HI. Pups (n = 41) were randomized in two experimental groups independently of sex and weight: LPS + HI (n = 21) and