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Regular Article

Pharmacologically induced hypothermia attenuates traumatic brain injury in neonatal rats

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ABSTRACT

Neonatal brain trauma is linked to higher risks of mortality and neurological disability. The use of mild to moderate hypothermia has shown promising potential against brain injuries induced by stroke and traumatic brain injury (TBI) in various experimental models and in clinical trials. Conventional methods of physical cooling, however, are difficult to use in acute treatments and in induction of regulated hypothermia. In addition, general anesthesia is usually required to mitigate the negative effects of shivering during physical cooling. Our recent investigations demonstrate the potential therapeutic benefits of pharmacologically induced hypothermia (PIH) using the neurotensin receptor (NTR) agonist HPI201 (formerly known as ABS201) in stroke and TBI models of adult rodents. The present investigation explored the brain protective effects of HPI201 in a P14 rat pediatric model of TBI induced by controlled cortical impact. When administered via intraperitoneal (i.p.) injection, HPI201 induced dose-dependent reduction of body and brain temperature. A 6-h hypothermic treatment, providing an overall 2–3 °C reduction of brain and body temperature, showed significant effect of attenuating the contusion volume versus TBI controls. Attenuation occurs whether hypothermia is initiated 15 min or 2 h after TBI. No shivering response was seen in HPI201-treated animals. HPI201 treatment also reduced TUNEL-positive and TUNEL/NeuN-colabeled cells in the contusion area and peri-injury regions. TBI-induced blood–brain barrier damage was attenuated by HPI201 treatment, evaluated using the Evans Blue assay. HPI201 significantly decreased MMP-9 levels and caspase-3 activation, both of which are pro-apototic, while it increased anti-apoptotic Bcl-2 gene expression in the peri-contusion region. In addition, HPI201 prevented the up-regulation of pro-inflammatory tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6. In sensorimotor activity assessments, rats in the HPI201 treated group exhibited improved functional recovery after TBI versus controls. These data support that PIH therapy using our NTR agonist is effective in reducing neuronal and BBB damage, attenuating inflammatory response and detrimental cellular signaling, and promoting functional recovery after TBI in the developing brain, supporting its potential for further evaluation towards clinical development.

Introduction

Traumatic brain injury (TBI) is a common clinical disorder in neonates and young children. TBI can cause significant brain damage involving neuronal cell death, gliosis, blood–brain barrier disruptions, brain edema, ischemia, inflammation and other pathological events (Sharp et al., 2014). In the developing brain, TBI may also cause neonatal seizures and epilepsy due to the hyperexcitability of neurons and neural circuits, resulting in long-term functional impairments (Choe et al., 2012; Finnie, 2012). Unfortunately, effective treatments to prevent the pathological and functional deficits after neonatal TBI have not been developed.

Abbreviations: BBB, Blood–brain barrier; CCI, Controlled cortical impact; EB, Evans blue; EPO, Erythropoietin; HIE, Hypoxic ischemic encephalopathy; IACUC, Institutional Animal Care and Use Committee; IFN-β, Interferon-β; IL-1β, Interleukin-1β; IL-6, Interleukin-6; i.p., Intraperitoneal; i.v., Intravenously; MMP-9, Matrix metalloproteinase-9; mPTP, Mitochondrial permeability transition pore; NO, Nitric oxide; PIH, Pharmacologically induced hypothermia; P14, Post-natal day 14; TBI, Traumatic brain injury; TNF-α, Tumor necrosis factor-α; TRP, Transient receptor potential; TUNEL, Terminal deoxynucleotidyl transferase dUTP nick end labeling

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Mild to moderate hypothermia has shown strong protective effects in both pre-clinical and clinical studies. Therapeutic hypothermia using physical cooling methods has been studied as treatments for a number of brain and peripheral organ disorders, including ischemic and hemorrhage strokes (Sheng et al., 2012; Wu and Grotta, 2013), epileptic seizures (Motamedi et al., 2013; Srinivasakumar et al., 2013), spinal cord injury (Ahmad et al., 2014), perinatal asphyxia (Rey-Funes et al., 2013) and others. In contrast, the potential benefits of hypothermic therapy for the treatment of TBI, especially in neonates and children, have been much less investigated.

Since conventional cooling methods such as the use of ice or surface cooling pads are not efficient (Jacobs et al., 2013), new methods in hypothermia therapy have been developed. These include epidural placement of cooling catheter (Inoue et al., 2012), passive heat dissipation (D'Ambrosio et al., 2013), local cold fluid infusion (Chen et al., 2013) and extracorporeal veno-venous blood cooling (Kuboi et al., 2013; Testori et al., 2013). Recently, pharmacologically induced hypothermia (PIH) or drug-induced hypothermia (DIH) has drawn increased attention due to its target specific, receptor/channel mediated effect and effective inductions of regulated hypothermia (Muzzi et al., 2013; Tupone et al., 2013; Zhang et al., 2013). For example, compounds acting at adenosine A1 receptors, opioid receptors, transient receptor potential (TRP) channels, and dopamine receptors can induce hypothermic effects (Muzzi et al., 2013; Tupone et al., 2013; Zhang et al., 2013). In mechanisms of pharmacological hypothermia, the hypothalamic thermoregulatory set point or peripheral temperature sensitive channels are affected (Katz et al., 2012); (Chang et al., 2013). Using our second generation neurotensin receptor (NTR) agonists we recently demonstrated dose-dependent regulatory hypothermia in the mouse and rat. These PIH compounds showed protective effects against brain injury and improved functional recovery after ischemic or hemorrhage stroke and TBI in adult animals (Choi et al., 2012; Wei et al., 2013). The NTR compound-induced neuroprotection is likely due to its hypothermic effect because when the animal body temperature was kept at normal level (36–37 °C) the protective effect of NTR compounds disappeared (Choi et al., 2012; Wei et al., 2013).

In the present investigation, we tested the hypothesis that pharmacological hypothermia can protect against TBI-induced brain injury in neonatal rats. The brain protective effect was examined at the molecular, cellular and tissue level as well as through functional testing, which provides a foundation for further evaluation of its potential for clinical development.

Materials and methods

Animals and trauma brain injury model

Neonatal Wistar rats at post-natal day 14 (P14) were subjected to controlled cortical impact insult. Pathological and behavioral changes were examined different days after TBI. The rats were housed in standard cages in 12 h light/12 h dark cycle. The animal protocol was approved by the Emory University Institutional Animal Care and Use Committee (IACUC), in compliance with National Institutes of Health (NIH) guidelines. The experimental TBI procedures were performed as previously described with minor modifications. P14 rats were anesthetized with 1.5% isoflurane and placed on a stereotaxic frame. After a midline skin incision, a 3.5 mm circular craniotomy was performed midway between lambda and bregma, 2.0 mm to the right of central suture using an electric drill. Controlled cortical impact was induced with an electric impact device using an impact tip. The PCI3000 precision cortical impactor (Hatteras Instruments, Cary, NC) and a 3.0 mm diameter flat fact tip with a slightly rounded edge (velocity = 3 m/s, depth = 2.0 mm, and contact time = 150 ms). Temperature was monitored by a rectal thermometer in all groups and maintained at 36 ± 0.5 °C during surgery, using a heating pad controlled by a homeothermic blanket control unit (Havard Apparatus, Holliston, MA, USA). After the injury, the skin was glued, and rats were allowed to recover in a humidity-controlled incubator (Thermocare, Incline Village, NV, USA). Animals in the sham and TBI groups were injected with saline after TBI, and their body temperature was maintained at 36–37 °C in a humidity-controlled incubator for up to 6 h after TBI. In the hypothermia group, animals were subjected to HPI201 and no other intervention of temperature change was applied.

Drug administration and hypothermia induction

Animals were randomly divided into three groups: Sham control, TBI plus saline vehicle control and TBI plus HPI201-induced hypothermia group. HPI201, dissolved in saline, was intraperitoneally (i.p.) injected. The first bolus injection (2 mg/kg) was given 15 min after TBI followed by additional one or two injections at a half of the initial dose (1 mg/kg, 1.5-2 h interval) to keep a constant mild hypothermia (32–35 °C) for 6 h.

Temperature measurement

Rectal and brain temperatures were measured during and after injury. Rectal temperature was monitored using a rectal probe (Harvard Apparatus) and measurements were repeated every 15 min for the first hour and every 60 min thereafter. Brain temperature was measured using a Physitemp probe (Temperature Data Acquisition System, Physitemp, Clifton, NJ) placed on the surface of the cerebral cortex. The Physitemp system allows simultaneous monitoring and data acquisition from seven animals either during or after anesthesia. The implantable thermocouple probe has a tip diameter of 0.16 mm. The microprobe was connected to a guide cannula that attached to the caps of two dummy cannulas (model C313 DC, Plastic Products Co., Roanoke, VA, USA). A small hole was drilled into the caps of a size sufficient to accommodate the shaft of the microprobe. The microprobe was then inserted through caps, positioned, and glued into place with epoxy resin. The cap closer to the needle tip was used to screw the modified probe into an implanted guide cannula; the other cap provided additional mechanical strength. The probe was connected with an extension wire to the data acquisition system controlled by a computer.

Quantification of contusion volume

To measure the contusion volumes, Nissl staining was performed on the brain sections collected at different time points after TBI. The rat brains were harvested and cut into 10 μM-thick fresh-frozen brain sections with 200 μm intervals. Sections were then fixed with a 1:1 mixture of 10% formalin and acetic acid for 10 min. After washing with distilled water for 5 min, slices were stained with a working solution containing buffer solution (0.1 M acetate acid and 0.1 M sodium acetate. 94:6) and Cresyl Violet acetate at a ratio of 5:1. The sections were then dehydrated in 100% ethyl alcohol and mounted.

After Nissl staining, the sections were digitized using ImageJ software (NIH, Bethesda, MD, USA), and the areas of the contusion tissue and the two hemispheres quantified by an investigator blinded to the treatment of the animals (n = 5–8 per group). Contusion volume was assessed based on the Cavalieri method as previously described (Lee et al., 2014). The number of sections and the section thickness (10 μm) were multiplied by the mean area of the remaining cortex. Contusion volumes were calculated based on the contusion areas (C) obtained from 10 to 12 sections as follows: C1*D + 0.2(C1 + C2 + 3 + …C12), with D being the distance between two sections (0.2 mm). Additionally, to consider the effects of swelling or edema, hemispheric tissue loss was calculated as a percentage calculated by [(contralateral hemispheric volume-ipsilateral hemispheric volume)/(contralateral hemispheric volume) × 100%]. The calculated result is reported as the ratio of lesion volume against the corrected hemisphere volume.