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ACUTE AND DELAYED PROTECTIVE EFFECTS OF PHARMACOLOGICALLY INDUCED HYPOTHERMIA IN AN INTRACEREBRAL HEMORRHAGE STROKE MODEL OF MICE

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Abstract—Hemorrhagic stroke, including intracerebral hemorrhage (ICH), is a devastating subtype of stroke; yet, effective clinical treatment is very limited. Accumulating evidence has shown that mild to moderate hypothermia is a promising intervention for ischemic stroke and ICH. Current physical cooling methods, however, are less efficient and often impractical for acute ICH patients. The present investigation tested pharmacologically induced hypothermia (PIH) using the second-generation neurotensin receptor (NTR) agonist HPI-201 (formerly known as ABS-201) in an adult mouse model with ICH. Acute or delayed administrations of HPI-201 (2 mg/kg bolus injection followed by 2 injections of 1 mg/kg, i.p.) were initiated at 1 or 24 h after ICH. HPI-201 induced mild hypothermia within 30 min and body and brain temperatures were maintained at 32.7 ± 0.4 °C for at least 6 h without causing observable shivering. With the 1-h delayed treatment, HPI-201-induced PIH significantly reduced ICH-induced cell death and brain edema compared to saline-treated ICH animals. When HPI-201-induced hypothermia was initiated 24 h after the onset of ICH, it still significantly attenuated brain edema, cell death and blood–brain barrier breakdown. HPI-201 significantly decreased the expression of matrix metallopeptidase-9 (MMP-9), reduced caspase-3 activation, and increased Bcl-2 expression in the ICH brain. Moreover, ICH mice received 1-h delayed HPI-201 treatment performed significantly better in the neurological behavior test 48 h after ICH. All together, these data suggest that systemic injection of HPI-201 is an effective hypothermic strategy that protects the brain from ICH injury with a wide therapeutic window. The protective effect of this PIH therapy is partially mediated through the alleviation of apoptosis and neurovascular damage. We suggest that pharmacological hypothermia using the newly developed neurotensin analogs is a promising therapeutic treatment for ICH.

Key words: intracerebral hemorrhage, pharmacological hypothermia, PIH, neurotensin receptor, ABS-201, HPI-201.

INTRODUCTION

Stroke is a leading cause of human death and disability in the US and across the world (Baldwin et al., 2010). Intracerebral hemorrhage (ICH) comprises 10–15% of all stroke cases. Patients suffering from ICH often have abysmal outcomes which are worse than ischemic stroke, with 30-day mortality estimated as high as 44%, and survivors typically suffer life-limiting disability (Broderick et al., 1993). Despite over three decades of research, the only FDA approved therapy for acute stroke is the tissue plasminogen activator (tPA), which is limited to ischemic stroke with a narrow therapeutic window of only 4.5 h (Baldwin et al., 2010). To date, there has been no effective drug therapy for hemorrhagic stroke. Several clinical trials of potential treatments for hemorrhagic stroke to prevent hematoma expansion, control blood pressure, and remove clots have proven unsuccessful or induced inconsistent results (Broderick et al., 1993; Sutherland et al., 2008; Diringer et al., 2010). Effective therapies for this catastrophic subtype of stroke are urgently needed.

Mild to moderate hypothermia, also known as therapeutic hypothermia, that reduces body temperature by 3–5 °C, is neuroprotective in pre-clinical and clinical studies for ischemic and hemorrhagic stroke subtypes (Hu et al., 2008; Theodorsson et al., 2008; Zhang et al., 2008; Torok et al., 2009; Fingas et al., 2009). Therapeutic hypothermia protects the brain via multiple mechanisms. It reduces necrotic and apoptotic cell death (Maier et al., 1998), attenuates intracranial pressure (Schwab et al., 1998), increases cerebral perfusion pressure (Schwab et al., 1998), alleviates

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Abbreviations: BBB, blood–brain barrier; CNS, central nervous system; INTERACT, Intensive Blood Pressure Reduction in Acute Cerebral Hemorrhage; ICH, intracerebral hemorrhage; MMP-9, matrix metallopeptidase-9; NSS, neurological stroke scale; NT, neurotensin; NTR, neurotensin receptor; PIH, pharmacologically induced hypothermia; PBS, phosphate-buffered saline; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; rTdT, terminal deoxynucleotidyl transferase recombinant enzyme; tPA, tissue plasminogen activator; TBS-T, Tween in Tris-buffered saline.

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brain edema (Thome et al., 2005; Linares and Mayer, 2009), decreases inflammatory cytokine secretion (Sutcliffe et al., 2001; Yanagawa et al., 2002; Han et al., 2003), and improves the overall cerebral metabolic status in part by modulating ATP depletion (Linares and Mayer, 2009). Additionally, therapeutic hypothermia reduces the risk of hemorrhagic conversion and protects against hemorrhage-induced brain damage including fever that often accompanies ICH (Thome et al., 2005; Linares and Mayer, 2009). Clinically, hypothermia has been considered an approved therapy for patients after cardiac arrest and in children with hypoxic-ischemic encephalopathy (Nagel et al., 2008). In a recent pilot study on 12 patients with large spontaneous ICH, treatment using physical cooling-induced mild hypothermia (35 °C) for 10 days prevented peri-hemorrhagic edema and improved 90-day survival (Kollmar et al., 2010). Another pilot study on 24 hemorrhagic stroke patients (8 in the hypothermic group and 18 in the control group) showed that mild hypothermia (34 °C for 24 h, within 48 h after stroke) resulted in statistically significant improvement at 0.5- and 1-year follow up examinations using the modified Rankin Scale score (Abdullah and Husin, 2011). In a more recent clinical study, 25 patients with large supra-tentorial ICH (volume > 25 ml) were treated with mild hypothermia of 35 °C for 8–10 days (Staykov et al., 2013). The body temperature in this trial was controlled using endovascular cooling catheters. Compared to a historical group of 25 patients with large ICH, peri-hemorrhagic edema volumes in the hypothermia group remained stable. Mortality rate was 8.3% in the hypothermia group versus 16.7% in the control group after 3 months and 28% versus 44 % after 1 year. This study suggests that mild hypothermia prevents the development of edema and reduces mortality rate in patients with large hemorrhagic stroke (Staykov et al., 2013). More active and coordinated efforts have been made to initiate a number of pilot studies on therapeutic hypothermia and a pan-European multicenter randomized controlled trial is underway (van der Worp et al., 2010).

A major dilemma that has limited the clinical application of therapeutic hypothermia in stroke patients is the inefficient and often impractical methods of physical cooling currently used to reduce body/brain temperatures. Physical cooling triggers undesirable shivering and vasoconstriction, both events make reduction and accurate control of temperature very challenging (Schwab et al., 1998; Staykov et al., 2013). With most advanced therapeutic hypothermia protocols, patients are admitted to the intensive care unit, typically sedated and mechanically ventilated. This rigorous approach exposes patients to multiple procedures and increased risk of side effects. In the search for a more efficient and practical hypothermia therapy, we have turned to a new concept of ‘‘regulated hypothermia’’ pharmacologically targeting central ‘‘thermoreceptors’’ and reducing the ‘‘set-point’’ in the thermoregulatory center, located in the hypothalamus (Katz et al., 2004). To this end, we have synthesized a group of neurotensin receptor (NTR) agonists that showed marked effect in reducing body and brain temperatures in rodents and monkeys (Tyler et al., 1999; Fantegrossi et al., 2005). We refer to this approach as pharmacologically induced hypothermia (PIH) or pharmacological hypothermia and have recently reported neuroprotective effects of the novel neurotensin analog HPI-201 (formerly ABS-201) against ischemic stroke in mice (Choi et al., 2012).

Neurotensin (NT) is a tri-decapeptide found in the central nervous system (CNS). It is a well-accepted regulator of central temperature control as well as analgesia through its interaction with NTRs (Tyler-McMahon et al., 2000; Feifel et al., 2010). Neurotensin itself does not normally cross the blood–brain barrier (BBB) and is rapidly degraded by peptidases. There are two major subtypes of neurotensin receptors, NTR1 and NTR2; both are G protein-coupled receptors. NTR1 activation is associated with lowering of body temperature and induction of analgesia. The clinical efficacy of first-generation neurotensin analogs was limited by their inability to penetrate the BBB. Additionally, oral preparations lacked stability which further limited their utility (Dubuc et al., 1992). Later pharmaceutical research has determined that the 8–13 fragment of neurotensin [NT(8–13)] is the active moiety, and second-generation neurotensin analogs that are biologically stable and can penetrate the BBB have been developed by the Dix’s group (Kokko et al., 2005). NT[8–13] analogs were initially developed as potential antipsychotics (Hadden et al., 2005) and analgesics (Hughes et al., 2010), but were also noted for their hypothermic effect mediated through NTR1 in the brain (Dubuc et al., 1999). Our novel neurotensin derivatives such as HPI-201 have the following chemical structure: CH3-homolys-Arg-Pro-Tyr-*tert*Leu-Leu-COOH (Hadden et al., 2005). They cross the BBB, show high affinity for human NTR1 and effectively induce regulated hypothermia (Hadden et al., 2005; Orwig et al., 2009). HPI-201 induces regulated hypothermia by intravenous, intraperitoneal or oral administration (Hadden et al., 2005). Along with our previous investigation of the PIH therapy in ischemic stroke (Choi et al., 2012), the present study is the first effort to test the second-generation hypothermic compound HPI-201 for early and delayed treatments of ICH in a rodent model. These investigations support that PIH therapy using novel compounds such as HPI-201 provides an effective and clinically feasibly approach after both ischemic and hemorrhagic strokes.

EXPERIMENTAL PROCEDURES

Animals

C57/BL6 male mice, 10-weeks-old and approximately 25 g in body weight, were purchased from the Jackson Laboratories (Bar Harbor, ME, USA). Animals were housed at room temperature with a 12-h light/dark cycle in the pathogen-free Laboratory Animal Center for Research at the Emory University. All the experimental