Neurobiology of Aging 38 (2016) 127–140

Contents lists available at ScienceDirect

Neurobiology of Aging

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

Twenty-four hours hypothermia has temporary efficacy in reducing brain infarction and inflammation in aged rats

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ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form 19 October 2015
Accepted 11 November 2015
Available online 1 December 2015

Keywords:
Aging
Stroke
Hypothermia
H_2S
Phagocytosis
Inflammation
Neurogenesis
EEG
Sleep
Neuroprotection

ABSTRACT

Stroke is a major cause of disability for which no neuroprotective measures are available. Age is the principal nonmodifiable risk factor for this disease. Previously, we reported that exposure to hydrogen sulfide for 48 hours after stroke lowers whole body temperature and confers neuroprotection in aged animals. Because the duration of hypothermia in most clinical trials is between 24 and 48 hours, we questioned whether 24 hours exposure to gaseous hypothermia confers the same neuroprotective efficacy as 48 hours exposure. We found that a shorter exposure to hypothermia transiently reduced both inflammation and infarct size. However, after 1 week, the infarct size became even larger than in controls and after 2 weeks there was no beneficial effect on regenerative processes such as neurogenesis. Behaviorally, hypothermia also had a limited beneficial effect. Finally, after hydrogen sulfide–induced hypothermia, the poststroke aged rats experienced a persistent sleep impairment during their active nocturnal period. Our data suggest that cellular events that are delayed by hypothermia in aged rats may, in the long term, rebound, and diminish the beneficial effects.

1. Introduction

Despite encouraging results from experiments with young animals, human stroke trials of neuroprotective factors which could be indicated after thrombolysis to limit infarct expansion and promote tissue recovery have not yielded satisfactory results (Sacco et al., 2007). One possible explanation for this discrepancy between laboratory and clinical outcomes is the role that age plays in the recovery of the brain from insult (Popa-Wagner et al., 2011). In this regard, the aged postacute animal model is clinically most relevant to stroke rehabilitation (Badan et al., 2003; Buchhold et al., 2007; Lucke-Wold et al., 2014; Petcu et al., 2010).

Aging brain reacts strongly to ischemia-reperfusion injury with an early inflammatory response. The process of cellular senescence can be an important additional contributor to chronic poststroke by creating a "primed" inflammatory environment in the brain. Overall, these proinflammatory reactions promote early scar formation associated with tissue fibrosis and reduce functional recovery (Buga et al., 2013).

To minimize the incapacitating sequelae of stroke, a promising focus of research is on long-term neuroprotective strategies that minimize functional impairment by preventing the death of neurons, which continues for days to weeks after focal cerebral ischemia.

A viable alternative to conventional drug-based therapies is physical cooling, or hypothermia, either confined to the head or including the entire body (Esposito et al., 2014; Hennerici et al., 2013; Kollmar et al., 2007; Wu and Grotta, 2013).

The feasibility of hypothermia (either by surface or endovascular cooling) has been addressed by several studies both in traumatic brain injury (for a review, see Dietrich and Bramlett, 2010) and stroke patients. Stroke patients were exposed for 6–24 hours to mild hypothermia (in the range, 33 °C–35.5 °C). Hypothermia was well-tolerated but its clinical benefits are

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http://dx.doi.org/10.1016/j.neurobiolaging.2015.11.006

limited, especially in the long term as measured by the National Institutes of Health Stroke Scale scores (Hemmen et al., 2010; Kammersgaard et al., 2000; Wan et al., 2014). However, one study reported that the infarct volume was lower in hypothermia patients than in nonhypothermia patients (De Georgia et al., 2004). More recently, it was shown that patients with ischemic stroke who underwent mild hypothermia after recanalization, had less cerebral edema and showed improved clinical outcome (Hong et al., 2014; Piironen et al., 2014).

The feasibility and clinical outcome after longer exposure to hypothermia (24 hours) is ongoing in a large, multicentre, phase 3, randomized trial funded by the European Union (EuroHYP-1). In this study, researchers are attempting to mirror the significant improvement of clinical outcomes noted after reanimation in patients with cardiac arrest. EuroHYP-1 participants are randomly assigned to either hypothermia and medical treatment or best medical treatment alone for acute ischemic stroke (van der Worp et al., 2014).

Methods to achieve surface cooling include water mattresses, alcohol bathing, and ice packing or more recently, using convective air blankets. Although feasible, maintenance of a constant body temperature using these approaches is difficult mainly because of temperature-dependent redistribution of blood flow due to vasoconstriction of superficial vasculature. Therefore, a simple method to pharmacologically induce long-term, regulated lowering of whole body temperature is highly desirable.

The concept of drug-induced cooling comes from the phenomenon of hibernation in some mammalian species which hibernate in a self-created H_2S environment, which results in lowered body temperature and slower metabolism (Blackstone et al., 2005). Experimentally, H_2S, a weak, reversible inhibitor of oxidative phosphorylation, induces a suspended animationlike state in mice by lowering body temperature to 15 °C during an exposure time of 6 hours at an ambient temperature of 13 °C (Blackstone et al., 2005). Using a modified version of this procedure, we previously showed that poststroke exposure of aged rats to H_2S-induced hypothermia for 48 hours resulted in a 50% reduction in infarct volume without causing obvious neurological or physiological side-effects (Florian et al., 2008; Joseph et al., 2012).

Therapeutic hypothermia has yielded inconsistent results with regards to the relationship between the depth of cooling and its effectiveness to reduce infarct volume and improve behavioral recovery. Thus, a systematic study showed that cooling at 34 °C over 4 hours after stroke was superior to other temperatures in the range 32 °C–37 °C (Huh et al., 2000; Kollmar et al., 2007; Maier et al., 1998). Therefore, prolonged hypothermia (at least 24 hours) and a longer survival time (at least 2 weeks) is a better study design to test the efficacy of hypothermia in experimental models and clinical trials. Along this line, prolonged cooling (33 °C for 24 hours and then 35 °C for 24 hours) started at 2.5 hours after reperfusion prevented the contralateral limb impairment in food pellet retrieval and reduced the infarct volume by 40% in an experimental model (Colbourne et al., 2000).

Efficient neuroprotection requires a long-term regulated lowering of whole body temperature. Previously, we reported that 48 hours poststroke exposure to H_2S effectively lowers whole body temperature and confers neuroprotection in aged animals (Florian et al., 2008; Joseph et al., 2012). Because the duration of hypothermia in most clinical trials was between 24 and 48 hours, and to avoid the complications associated with longer exposure to hypothermia often seen in humans, we asked if a 24-hour exposure to gaseous hypothermia, within the optimal hypothermia depth of 32 °C, has the same neuroprotective efficacy as a 48 hours exposure.

2. Materials and methods

2.1. Animals and experimental design

The subjects of these experiments were aged male Sprague-Dawley rats (18–20 months of age; 550–647 g) kept under standard laboratory conditions with free access to food and water (except under specific conditions, in the following). Animals were assigned to 2 groups: group 1 (N = 34) with middle cerebral artery occlusion (MCAO); and group 2 (N = 37) with MCAO plus hypothermia. Eight animals served as sham controls (N = 8). All experiments were approved by the Animal Experimentation Ethics Board of the State of Mecklenburg-Vorpommern as meeting the ethical requirements of the German National Act on the Use of Experimental Animals and by Experimentation Ethics Board of the Medical University of Craiova as meeting the ethical requirements of the European Union legislation on the protection of animals used for experimental purposes.

2.2. Reversible occlusion of the middle cerebral artery

Eighteen hours before surgery, the rats were deprived of food to minimize variability in ischemic damage that can result from varying plasma glucose levels. Water remained available at all times. In all cases, surgery was performed between 9:00 and 13:00.

Animals from the control and experimental groups were randomly subjected to cerebral infarction that was induced by the focal interruption of blood flow by transiently lifting the middle cerebral artery with a tungsten hook, as previously described (Popa-Wagner et al., 2010). Throughout surgery, anesthesia was maintained by spontaneous inhalation of 1%–1.5% isoflurane in a mixture of 75% nitrous oxide and 25% oxygen. Body temperature was maintained at 37 °C by a Homeothermic Blanket System (Harvard Apparatus), and the tail artery was catheterized to allow the continuous measurement of blood pressure and the withdrawal of blood samples for determination of pH and blood gases (Blutgassystem IL 1620, Instrumentation Laboratory, Munich), as well as arterial glucose levels (Omnican7 Balance, B. Braun, Melsungen). Local changes in blood flow were monitored using a laser Doppler device (Perimed, Stockholm, Sweden), and blood gases were measured at several time points during ischemia. A decrease in the laser Doppler signal to <20% of control values was considered to indicate successful MCA occlusion. After 90 minutes, the hook was released and the common carotid arteries were reopened.

2.3. Experimental H_2S-induced hypothermia

One hour after the resumption of blood flow, individual rats from the second group were exposed for 24 hours to an atmosphere containing 80 ppm H_2S and 19.5% O_2, achieved by mixing room-air with 5000 ppm H_2S-balanced nitrogen at a flow rate of 3 L/min, as previously described (Florian et al., 2008; Joseph et al., 2012). After 2 hours, the concentration of H_2S was reduced to 40 ppm (the toxicity threshold for H_2S is 80 ppm). The temperature outside the experimental box was maintained at 21 °C in a well-ventilated room. Water was available during this period, although no appetitive activity was observed. Carbon dioxide, oxygen, and H_2S were measured continuously using appropriate gas detectors (GfG, Dortmund, Germany) placed directly in the cage as previously described by our group (Florian et al., 2008).

2.4. Prolonged telemetric EEG recordings after H_2S hypothermia

Telemetric recordings of body temperature were carried out in 14 rats with MCAO (7 exposed to H_2S and 7 controls) using a CTA-F40