Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Post-ischemic intra-arterial infusion of liposome-encapsulated hemoglobin can reduce ischemia reperfusion injury

Daisuke Shimbo, Takeo Abumiya, Hideo Shichinohe, Naoki Nakayama, Ken Kazumata, Kiyohiro Houkin*

Department of Neurosurgery, Hokkaido University Graduate School of Medicine, Sapporo 060-8638, Japan

ARTICLE INFO

Article history:
Accepted 23 January 2014
Available online 31 January 2014

Keywords:
Ischemia reperfusion injury
Artificial oxygen carrier
Neutrophil
MMP-9

ABSTRACT

Despite successful revascularization, reperfusion after prolonged ischemia causes ischemia reperfusion (I/R) injury. Recruitment and activation of neutrophils is thought to be a key event causing I/R injury. We examined whether post-ischemic intra-arterial infusion of liposome-encapsulated hemoglobin (LEH), an artificial oxygen carrier without neutrophils, could reduce I/R injury in a rat transient middle cerebral artery occlusion (MCAO) model. Male Sprague-Dawley rats were subjected to 2-h MCAO and then were divided into three groups: (1) LEH group ($n=7$) infused with LEH (Hb concentration of 6 g/dl, 10 ml/kg/h) through the recanalized internal carotid artery for 2 h, (2) vehicle group ($n=8$) infused with saline (10 ml/kg/h) in the same manner as the LEH group, and (3) control group ($n=9$) subjected to recanalization only. After 24-h reperfusion, all rats were tested for neurological score and then sacrificed to examine infarct and edema volumes, myeloperoxidase (MPO) expression, matrix metalloproteinase-9 (MMP-9) expression and activity, and reactive oxygen species (ROS) production. Compared with the control group and the vehicle group, the LEH group showed a significantly better neurological score and significantly smaller infarct and edema volumes. MPO expression, MMP-9 expression and activity, and ROS production in the LEH group were also significantly lower than those in the control and vehicle groups. The results in the present study suggest that post-ischemic intra-arterial infusion of LEH can reduce I/R injury through reducing the effect of MMP-9, most likely produced by neutrophils. This therapeutic strategy may be a promising candidate to prevent I/R injury after thrombolysis and/or thromboectomy.

1. Introduction

Intravenous thrombolysis with tissue plasminogen activator (tPA) and mechanical thromboectomy are effective treatments to achieve recanalization in ischemic stroke. Recanalization is the most important action to obtain a favorable outcome in ischemic stroke patients. However, recently, it has been demonstrated that a couple of clinical trials of ischemic stroke

Abbreviations: LEH, liposome-encapsulated hemoglobin; I/R, ischemia reperfusion; MPO, myeloperoxidase; MMP-9, matrix metalloproteinase-9; ROS, reactive oxygen species; MCAO, middle cerebral artery occlusion

*Corresponding author. Fax: +1 81 11 708 7737.
E-mail address: abumiya@med.hokudai.ac.jp (T. Abumiya).

0006-8993/$ - see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2014.01.038

treated with mechanical thromboectomy with or without tPA therapy failed to show a favorable outcome (Broderick et al., 2013; Ciccone et al., 2013; Kidwell et al., 2013). Despite successful revascularization after treatment, reperfusion after prolonged ischemia causes infarct enlargement with severe brain edema and hemorrhagic transformation rather than tissue recovery due to so-called "ischemia reperfusion (I/R) injury" Molina and Alvarez-Sabín (2009). It is necessary to develop novel treatments for I/R injury to prevent brain edema and hemorrhagic transformation.

The mechanism of I/R injury has been extensively studied using animal transient focal ischemic models (Wong and Crack, 2008; Pan et al., 2007). Experimentally, focal I/R induces recruitment and activation of inflammatory cells, which are thought to be key events causing I/R injury, represented by blood brain barrier (BBB) breakdown brain edema, neural death, and hemorrhagic transformation (Amantea et al., 2009; Jin et al., 2010). Many studies have shown that systemic suppression of neutrophils attenuated I/R injury in a transient middle cerebral artery occlusion (MCAO) model (Matsuo et al., 1994; Mori et al., 1992; Zhang et al., 1994; Connolly et al., 1996).

Although suppression of neutrophils would be an attractive therapeutic strategy for I/R injury, systemic suppression may induce adverse effects on the defense mechanism against infection. We therefore developed a new therapeutic approach by reducing neutrophil influx into the I/R region. For this purpose, we used liposome-encapsulated hemoglobin (LEH), which was developed as an artificial oxygen carrier. LEH is composed of liposome capsules containing purified human hemoglobin without any blood cells, including neutrophils. We examined whether post-ischemic selective intra-arterial infusion of LEH to reduce localized inflow of neutrophils can ameliorate I/R injury in a rat transient MCAO model.

2. Results

2.1. Physiological parameters

Physiological parameters were monitored twice at the beginning of MCAO and the end of reperfusion. There was no statistically significant difference in the physiological parameters among the three groups, as shown in Table 1.

2.2. Neurological score

The rats in the three groups were re-evaluated using their Bederson score after 24-h reperfusion. While rats in the control and vehicle groups showed no improvements and retained a 3-point Bederson score, some rats in the LEH group showed score improvements. The scores were significantly better in the LEH group (2.51±0.49) than in the control group (3.0±0) and in the vehicle group (3.0±0) (Fig. 1).

2.3. Infarct and edema volumes

Brain infarct and edema volumes after 24-h reperfusion were measured using 2,3,5-triphenyltetrazolium chloride (TTC) staining. Infarct volume of the LEH (34.3±7.9%) group was significantly smaller than that of the control (51.4±5.2%) and vehicle (49.6±6.4%) groups. Edema volume of the LEH group (114.6±4.8%) was significantly smaller than that of the control (121.8±5.8%) and vehicle (121.3±9.4%) groups (Fig. 2).

Table 1 – Physiological parameters of each group.

	Control	Vehicle	LEH
Pre MCAO			
Body temperature (C)	36.6±0.32	36.4±0.24	36.5±0.34
MABP (mmHg)	96.8±4.8	97.1±2.9	99.4±7.0
pH	7.49±0.04	7.50±0.03	7.50±0.03
pCO_2 (mmHg)	35.3±3.6	32.0±5.6	33.4±4.3
pO_2 (mmHg)	139±8.4	140±11.4	133±7.87
Ht (%)	41.0±3.9	37.1±3.4	41.2±2.9
After 24 h reperfusion			
pH	7.46±0.04	7.52±0.04	7.49±0.05
pCO_2 (mmHg)	33.8±2.5	30.7±4.0	33.3±4.4
pO_2 (mmHg)	130±8.4	134±12.3	138±13.8
Ht (%)	42.8±1.6	42.4±3.3	42.1±3.6

All values are the mean±SD. MABP: mean arterial blood pressure.

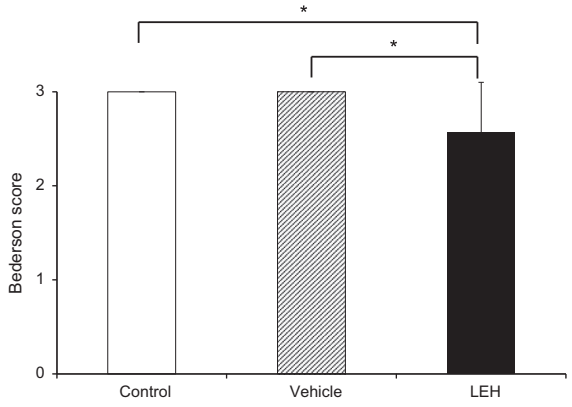

Fig. 1 – Effect of intra-arterial infusion of LEH on neurological function. Neurological function was evaluated by the Bederson score after 24-h reperfusion. The bar graph shows the Bederson score of each group. *$p<0.05$.

2.4. Myeloperoxidase (MPO) Western blotting

MPO Western blotting was performed to assess neutrophil infiltration into the I/R region. MPO expression in the LEH group was significantly lower than that in the control and vehicle groups (Fig. 3). MPO expression levels in the LEH group were less than 20% of those in the control group. MPO expression in the vehicle group was somewhat lower than that in the control group, but the difference was not statistically significant.

2.5. Matrix metalloproteinase-9 (MMP-9) Western blotting and zymography

The result of MMP-9 Western blotting showed a similar pattern to that of MPO Western blotting. MMP-9 expression in the LEH group was significantly lower than that in the