Brain Research 1651 (2016) 95–103

Contents lists available at ScienceDirect

Brain Research

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

Transarterial regional hypothermia provides robust neuroprotection in a rat model of permanent middle cerebral artery occlusion with transient collateral hypoperfusion

Kota Kurisu, Takeo Abumiya*, Masaki Ito, Masayuki Gekka, Toshiya Osanai, Hideo Shichinohe, Naoki Nakayama, Ken Kazumata, Kiyohiro Houkin

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

ARTICLE INFO

Article history:
Received 17 June 2016
Received in revised form
31 August 2016
Accepted 12 September 2016
Available online 20 September 2016

Keywords:
Apoptosis
Gliosis
Hypothermia
Partial reperfusion injury
Permanent middle cerebral artery occlusion
Transarterial regional hypothermia

ABSTRACT

The robust neuroprotective effects of transarterial regional hypothermia have been demonstrated in the typical transient middle cerebral artery occlusion (tMCAO) model, but have not yet been tested in other ischemic stroke models, even though clinical ischemic conditions are diverse. In order to clarify these effects in a different ischemic stroke model, we employed a rat model of permanent MCAO (pMCAO) with transient collateral hypoperfusion (tCHP), which was achieved by direct MCA ligation through craniotomy and 1-h bilateral common carotid artery occlusion at the beginning of pMCAO. The infusion of 20 ml/kg of 4 °C cold saline (CS) or 37 °C warm saline (WS) into the ipsilateral internal carotid artery (ICA) was performed for 15 min in intra- or post-tCHP. Neurological scores, infarct/edema volumes, and neuronal apoptosis and reactive gliosis were compared between the CS and WS groups and a non-infusion control group after 48 h of reperfusion. Although brain temperatures were only reduced by 2–3 °C for 15 min, the CS group had significantly better neurological scores, smaller infarct/edema volumes, and less penumbral neuronal apoptosis and reactive gliosis than the control and WS groups. The post-tCHP CS group exhibited prominent neuroprotective effects, even though infarct volumes and neuronal apoptosis were reduced less than those in the intra-tCHP CS group. In conclusion, we demonstrated the neuroprotective effects of transarterial regional hypothermia in an ischemic model of pMCAO with tCHP. Even though MCAO is persistent, cold infusion via the ICA is neuroprotective for the penumbra, suggesting the wider therapeutic application of this therapy.

1. Introduction

Although numerous experimental studies have demonstrated that hypothermia therapy exerts robust neuroprotective effects on ischemic stroke (Dumitrascu et al., 2016; Maier et al., 1998; van der Worp et al., 2007; Yenari and Han, 2012), clinical trials (Hemmen et al., 2010; Lyden et al., 2014) have failed to show any therapeutic benefit due to the adverse systemic influences accompanying this therapy (Esposito et al., 2014). Therefore, transarterial regional hypothermia is strongly expected to become a novel attractive treatment for acute ischemic stroke because of its rapid cooling action and fewer systemic side effects (Dumitrascu et al., 2016; Esposito et al., 2014; Kurisu et al., 2016). Consistent with previous findings (Chen et al., 2013; Ding et al., 2003, 2004a, 2004b; Luan et al., 2004; Zhao et al., 2009), we also clarified that transarterial regional hypothermia exerts strong neuroprotective effects on ischemia-reperfusion (I/R) injury (Kurisu et al., 2016). However, the effects of this therapy have only been examined in a typical ischemic stroke model, the transient middle cerebral artery occlusion (tMCAO) model (Chen et al., 2013; Ding et al., 2003, 2004a, 2004b; Kurisu et al., 2016; Luan et al., 2004; Zhao et al., 2009), which is a complete reperfusion model (Fluri et al., 2015; Takahashi et al., 2012; Zhao and Steinberg, 2011). The effects of this therapy have not yet been investigated in other ischemic stroke models, even though ischemic stroke in humans is extremely diverse in its pattern of occlusion and reperfusion in actual clinical settings. Therefore, we attempted to examine the therapeutic effects of transarterial regional hypothermia in a different ischemic stroke model (Fluri et al., 2015; Takahashi et al., 2012; Zhao and Steinberg, 2011).

In the present study, we investigated the neuroprotective effects of transarterial regional hypothermia on permanent MCAO (pMCAO) with transient collateral hypoperfusion (tCHP). In order to evaluate the effectiveness of transarterial regional hypothermia in terms of

* Corresponding author.
E-mail address: abumiya@med.hokudai.ac.jp (T. Abumiya).

http://dx.doi.org/10.1016/j.brainres.2016.09.017
0006-8993/

time interval and perfusion conditions, the treatment was performed in intra- or post-tCHP. We also assessed neuronal apoptosis and reactive gliosis by immunofluorescence staining to observe pathophysiological reactions in the ischemic core and penumbra.

2. Results

2.1. BraIn temperature and physiological parameters

Brain temperature was continuously monitored for 1 h during surgery in the intra-tCHP cold saline (CS), intra-tCHP warm saline (WS), and control groups (Fig. 1). It was rapidly and significantly lowered by the CS infusion in both the cortex (from 34.1 ± 1.2 °C to 32.5 ± 0.9 °C, $p < 0.01$) and striatum (from 36.4 ± 0.8 °C to 34.3 ± 1.0 °C, $p < 0.01$) during the CS infusion period. Since it was rapidly elevated after finishing the infusion, the brain temperature-lowering effect was only maintained for 15 min. Rectal temperature did not change during the observational period.

Physiological parameters were monitored twice: at the pre-ischemic baseline and the time of sacrifice (48 h after surgery). No significant differences were observed in physiological parameters between any of the five groups tested (Table 1).

2.2. Neurological function

Neurological function was examined in rats in each group using an 18-point scale score (Garcia et al., 1995) 48 h after surgery (Fig. 2A). Neurological scores were significantly better in the intra- and post-tCHP CS groups than in the control and intra- and post-tCHP WS groups ($p < 0.01$).

2.3. Infarct and edema formation

Representative images of brain sections stained with 2,3,5-triphenyltetrazolium chloride (TTC) are shown in Fig. 2B. Infarct volumes were significantly smaller in the intra-tCHP CS group ($8.1 \pm 4.7\%$, $p < 0.01$) and post-tCHP CS group ($15.7 \pm 5.9\%$, $p < 0.01$) than in the control and intra- and post-tCHP WS groups (~35%) (Fig. 2C). Edema volumes were also significantly smaller in the intra- and post-tCHP CS groups ($p < 0.01$) than in the control and intra- and post-tCHP WS groups (Fig. 2C). Comparisons between the intra- and post-tCHP CS groups revealed that infarct volumes were significantly smaller in the intra-tCHP CS group than in the post-tCHP CS group ($p < 0.05$) (Fig. 2C).

2.4. Appearance of neuronal apoptosis in the penumbra

Double immunostaining for cleaved caspase 3 (CC3) and NeuN was performed in order to observe cell apoptosis and viable neuronal cells in regions of interest (ROIs). Representative images of double NeuN and CC3 staining in the penumbra are shown in Fig. 4A. The frequent appearance of CC3-positive cells and a reduction in the number of NeuN-positive cells in the penumbra were observed in the control and intra- and post-tCHP WS groups, but less so in the intra- and post-tCHP CS groups (Fig. 3A). A quantitative analysis of CC3-positive cells revealed that apoptotic cell numbers were significantly less ($p < 0.01$) in the intra- and post-tCHP CS groups than in the control and intra- and post-tCHP WS groups (Fig. 3B). The number of viable neuronal cells was significantly higher ($p < 0.01$) in the intra- and post-tCHP CS groups than in the control and the intra- and post-tCHP WS groups (Fig. 3B). A comparison between the intra- and post-tCHP CS groups revealed that the suppressive effects of neuronal apoptosis were significantly stronger in the intra-tCHP CS group than in the post-tCHP CS group ($p < 0.01$). These suppressive effects by the CS infusion were not observed in the ischemic core (data not shown).

2.5. Appearance of reactive gliosis in the penumbra

Immunostaining for glial fibrillary acidic protein (GFAP) was performed in order to evaluate the activation of astrocytes. Representative images of GFAP staining in the penumbra are shown in Fig. 4A. The up-regulated expression of GFAP, representing glial activation, in the penumbra was observed in the control and intra- and post-tCHP WS groups, but not in the intra- or post-tCHP CS group (Fig. 4A). A quantitative analysis of the area of GFAP-positive staining revealed that it was significantly smaller ($p < 0.01$) in the intra- and post-tCHP CS groups than in the control and intra- and post-tCHP WS groups (Fig. 4B). Immunostaining for ionized calcium binding adapter molecule 1 (Iba1) was also performed in order to evaluate microglial activation. Representative images of Iba1 staining in the penumbra are shown in Fig. 5A. The up-regulated expression of Iba1, mainly amoeboid microglia, in the penumbra was observed in the control and intra- and post-tCHP WS groups, but not in the intra- or post-tCHP CS group (Fig. 5A). A quantitative analysis of the number of Iba1-positive cells revealed that it was significantly lower ($p < 0.01$) in the intra- and post-tCHP CS groups than in the control and intra- and post-tCHP WS groups. These suppressive effects on reactive gliosis by the CS infusion were not observed in the ischemic core (data not shown).

3. Discussion

The present study demonstrated that transarterial regional hypothermia exerts robust neuroprotective effects in the pMCAO with tCHP model, as well as the typical tMCAO model, as reported previously (Chen et al., 2013; Ding et al., 2003, 2004a, 2004b; Kurisu et al., 2016; Luan et al., 2004; Zhao et al., 2009). This therapy significantly improved neurological deficits and decreased

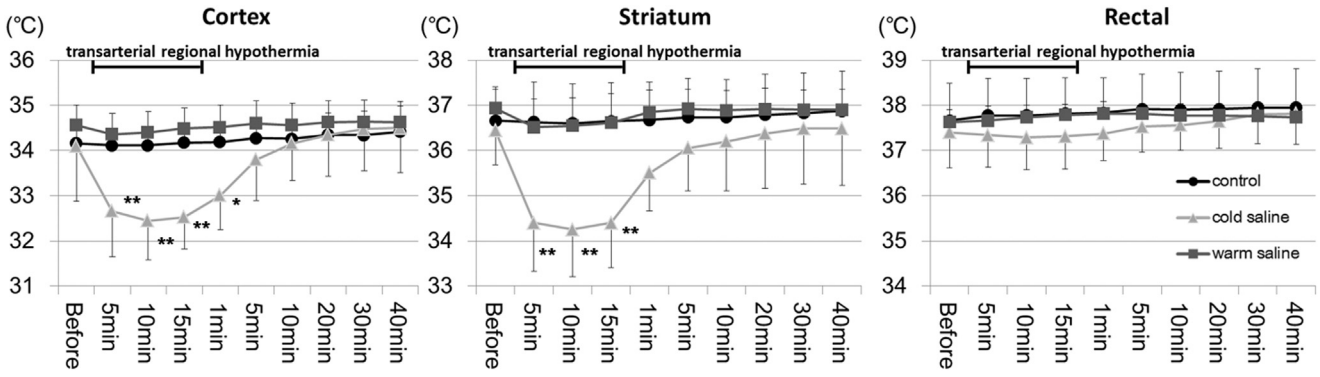

Fig. 1. Temperatures measured in the ipsilateral cortex, striatum, and rectum during and after transarterial regional hypothermia. *$p < 0.05$,**$p < 0.01$.