Neuroscience Letters 640 (2017) 70–75

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

Neurocardiac protection with milrinone for restoring acute cerebral hypoperfusion and delayed ischemic injury after experimental subarachnoid hemorrhage

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HIGHLIGHTS

- SAH can induce acute cerebral hypoperfusion that may lead to devastating outcome.
- Acute MIL increases CO to restore global CBF depression early after SAH.
- MIL prevents the occurrence of DCI and related neurobehavioral worsening.
- Neurocardiac protection with MIL promotes recovery from post-SAH early brain injury.

ARTICLE INFO

Article history:
Received 6 November 2016
Received in revised form
30 December 2016
Accepted 5 January 2017
Available online 6 January 2017

Keywords:
Cardiac output
Cerebral blood flow
Early brain injury
Mouse model
Milrinone
Delayed cerebral ischemia
Subarachnoid hemorrhage

ABSTRACT

Background and purpose: Acute cerebral hypoperfusion following subarachnoid hemorrhage (SAH) is highly related to the pathogenesis of delayed cerebral ischemia (DCI), but the therapeutic option is poorly available. This study aimed to clarify the effect of milrinone (MIL) on cerebral blood flow (CBF) and related outcomes after experimental SAH.
Methods: Twenty-seven male C57BL/6 mice were assigned to either sham surgery (SAH-sham; n = 6), SAH induced by endovascular perforation (control; n = 10), or SAH followed by cardiac support with intravenous MIL (n = 11) performed 1.5-h after SAH induction. CBF, neurobehavioral function, occurrence of DCI were assessed by MR-continuous arterial spin labeling, daily neurological score testing, and diffusion- and T2-weighted MR images on days 1 and 3, respectively.
Results: Initial global CBF depression was notable in mice of control and MIL groups as compared to the SAH-sham group ($P < 0.05$). MIL raised CBF in a dose-dependent manner ($P < 0.001$), resulted in lower incidence of DCI ($P = 0.008$) and better recovery from neurobehavioral decline than control ($P < 0.001$). The CBF values on day 1 predicted DCI with a cut-off of 42.5 ml/100 g/min (82% specificity and 83% sensitivity), which was greater in mice treated with MIL than those of control (51.7 versus 37.6 ml/100 g/min; $P < 0.001$).
Conclusion: MIL improves post-SAH acute hypoperfusion that can lead to the prevention of DCI and functional worsening, acting as a neurocardiac protective agent against EBI.

1. Introduction

Stroke is the leading cause of death and permanent disability across the world. Aneurysmal subarachnoid hemorrhage (SAH) can lead to devastating outcomes for patients in prime [1]. To date, symptomatic cerebral vasospasm or delayed cerebral ischemia (DCI) is the most popular and potentially treatable cause of secondary neurological injury after SAH, though it continues to be

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http://dx.doi.org/10.1016/j.neulet.2017.01.008
0304-3940/

a difficult clinical paradigm to treat with limited pharmacologic agents [2].

Several clinical and laboratory studies have shown that acute cerebral hypoperfusion [3] induced by post-hemorrhagic early brain injury/ischemia (EBI) is highly linked to the pathogenesis of DCI and associated adverse secondary outcomes [4,5]. One key factor underlying EBI is acute cerebral hypoperfusion [3], which can be the intriguing phenomenon of DCI. However, studies on the real-time changes of cerebral blood flow (CBF) in super-acute phase following SAH are poorly understood, therefore limiting the number of effective pharmaceutical treatment options.

In the clinical setting, we sometimes use inotropic hemodynamic augmentation with catecholamines in hopes of restoring focal cerebral ischemia attributable to DCI [6–9]. However, most popular inotropic agents such as dobutamine may provide limited effects on CBF early after SAH because they may aggravate cardiac failure due to massive stress hormone release caused by severe acute brain injury [10,11]. There are also concerns of cerebral vasoconstriction even with the use of the most reliable vasopressor norepinephrine [12].

The phosphodiesterase (PDE)-3 inhibitor milrinone is an option when inotropes are required but catecholamines are contraindicated due to tachycardia, left ventricular outflow obstruction or neurogenic cardiomyopathies [13–16]. In addition, a recent experimental study showed a concept of neurocardiac protection against EBI by brief inhalation of isoflurane delivered as a postconditioning therapeutic agent [17,18]. We hypothesized that application of milrinone to the isoflurane postconditioning strategy may have potential beneficial effects on EBI to restore and/or stabilize the CBF to prevent the occurrence of DCI after SAH. In the present proof-of-concept study, we sought to determine the impact of milrinone on acute hypoperfusion attributable to EBI and related outcomes using a non-invasive real-time CBF analysis with magnetic resonance imaging (MRI) in a mouse model of SAH.

2. Materials and methods

2.1. Animals

The protocols were approved by the Animal Care and Use Committee at our institute. All efforts were made to minimize the number of animals used and their suffering. A total of 27 male mice (C57BL/6N, age 7 weeks, weight 21–26 g) purchased from Charles River Laboratory Japan (Kanagawa, Japan). All animals were housed in an animal housing facility with temperature, humidity, and light (12-h light-12-h dark cycle) controlled. All animals were housed in groups of five per cage upon arrival with freely access to food and water and used at 9 weeks of age after 2-week acclimation period.

2.2. Experimental groups and animal preparation

Animals were assigned into 3 experimental groups: sham-operation (SAH-sham, n = 6), SAH followed by postconditioning using isoflurane inhalation (control, n = 10), and SAH followed by isoflurane postconditioning combined with intravenous MIL infusion (MIL, n = 11) (Fig. 1a). SAH was induced by endovascular perforation using a 5-0 monofilament suture at the terminal of the left internal carotid artery as described [19]. In sham-operated mice, the suture was introduced into the internal carotid artery, but without vessel perforation. Postconditioning was performed 1-h after SAH induction by a mask inhalation of 2% isoflurane in 50% air and 50% O_2 for 1.5-h [20,21].

2.3. MRI

In vivo MRI experiments were performed using a 4.7 T MRI scanner (Agilent Technologies, Santa Clara, CA). Mice were positioned prone in a plastic cradle with an integral head mask and the rectal temperature was maintained at $37 \pm 1\,^{\circ}C$ using heated air passed through the radiofrequency coil and a cylindrical plastic extension, by monitoring blood oxygen saturation (SpO_2) and pulse rate were monitored with a MRI-compatible pulse oximeter (MouseOx PLUS, Starr Life Sciences Corp., Oakmont, PA, USA). Whole-brain MRI was performed to ensure correct head positioning with acquisition of diffusion-weighted images (DWI) and T2-weighted images (T2WI) with fast spin echo sequences and 3-dimentional T2*WI with gradient echo sequences [19]. Continuous arterial spin labeling (CASL) was acquired with a gradient echo sequence [20,21].

2.4. Echocardiography

Doppler echocardiography was performed using the Vevo 3100 System (FUJIFILM VisualSonics SonoCite Inc., ON, Canada) for measuring transaortic velocities [17,20]. The product of peak velocity around the waveform at the aortic outflow and the cross-sectional area of the aortic valve allowed a measurement of stroke volume (SV). CO was calculated as the product of SV and heart rate.

2.5. Neurobehavioral test

Neurobehavioral function was assessed daily by a single, blinded observer (KN) using a modified Garcia score [22] consisted of the six tests evaluating both motor function and sensory performance (i.e., spontaneous activity, symmetry in the movement of four limbs, forepaw outstretching, wire cage climbing, body proprioception and response to vibrissae touch). The total score ranged from 3 to18 and a higher score indicated better performance.

2.6. Experimental protocols

After obtaining the baseline data of echocardiography followed by MRI during postconditioning, incremental doses (0.25 μg/kg/min; raised every 15-min interval) of intravenous MIL infusion starting from 0.25 μg/kg/min to 0.75 μg/kg/min were administered. Echocardiography was repeated for evaluating the cardiac parameters at each dose of MIL (0.25–0.75 μg/kg/min). CASL-CBF measurements were repeated every 3-min during the MIL challenge. Quantitative CBF analysis employed regions of interest including the whole brain on the basal ganglia section level of the reference image and the value for each dose at 12 min (0.25 μg/kg/min), 27 min (0.5 μg/kg/min), and 42 min (0.75 μg/kg/min) after starting MIL infusion has been analyzed. The same time points have been taken for mice of SAH-sham and control groups. After the MRI examination, echocardiography was repeated for evaluating the cardiac parameters at maximum MIL dose (0.75 μg/kg/min). Arterial blood gas measurements were performed twice at the beginning and end of postconditioning.

The animals were allowed to recover from anesthesia for 3 days for neurobehavioral tests. Following the final neurobehavioral tests on day 3, mice were anesthetized with isoflurane for MRI data acquisition. For assessment of DCI, presence of new infarction was assessed by hyperintense signals on T2WI on day 3 (Fig. 1a). After completion of final MRI examination, mice were euthanized by an overdose of pentobarbital (100 mg/kg) administered intraperitoneally.