Neurobiology of Disease 71 (2014) 292–304

Contents lists available at ScienceDirect

Neurobiology of Disease

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

Interplay between brain stem angiotensins and monocyte chemoattractant protein-1 as a novel mechanism for pressor response after ischemic stroke

Alice Y.W. Chang [a,b,c,d,*,1], Faith C.H. Li [a], Chi-Wei Huang [d,e], Julie C.C. Wu [a], Kuang-Yu Dai [a], Chang-Han Chen [a], Shau-Hsuan Li [f], Chia-Hao Su [a], Re-Wen Wu [g]

[a] Center for Translational Research in Biomedical Sciences, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan, ROC
[b] Department of Physiology, National Cheng Kung University, Tainan, Taiwan, ROC
[c] Department of Pharmacology, Kaohsiung Medical University, Kaohsiung, Taiwan, ROC
[d] Department of Biological Science, National Sun Yat-sen University, Kaohsiung, Taiwan, ROC
[e] Department of Neurology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan, ROC
[f] Department of Hematology and Oncology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan, ROC
[g] Department of Orthopedics, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan, ROC

ARTICLE INFO

Article history:
Received 4 January 2014
Revised 3 July 2014
Accepted 2 August 2014
Available online 12 August 2014

Keywords:
Ischemic stroke
Middle cerebral artery occlusion
Pressor response after stroke
Angiotensin isoforms and receptors
Monocyte chemoattractant protein-1
Neuroinflammation
Aliskiren
Rostral ventrolateral medulla

ABSTRACT

Pressor response after stroke commonly leads to early death or susceptibility to stroke recurrence, and detailed mechanisms are still lacking. We assessed the hypothesis that the renin–angiotensin system contributes to pressor response after stroke by differential modulation of the pro-inflammatory chemokine monocyte chemoattractant protein-1 (MCP-1) in the rostral ventrolateral medulla (RVLM), a key brain stem site that maintains blood pressure. We also investigated the beneficial effects of a novel renin inhibitor, aliskiren, against stroke-elicited pressor response. Experiments were performed in male adult Sprague–Dawley rats. Stroke induced by middle cerebral artery occlusion elicited significant pressor response, accompanied by activation of angiotensin II (Ang II)/type I receptor (AT1R) and AT2R signaling, depression of Ang-(1–7)/MasR and Ang IV/AT4R cascade, alongside augmentation of MCP-1/C–C chemokine receptor 2 (CCR2) signaling and neuroinflammation in the RVLM. Stroke-elicited pressor response was significantly blunted by antagonism of AT1R, AT2R or MCP-1/CCR2 signaling, and eliminated by applying Ang-(1–7) or Ang IV into the RVLM. Furthermore, stroke-activated MCP-1/CCR2 signaling was enhanced by AT1R and AT2R activation, and depressed by Ang-(1–7)/MasR and Ang IV/AT4R cascade. Aliskiren inhibited stroke-elicited pressor response via downregulating MCP-1/CCR2 activity and reduced neuroinflammation in the RVLM; these effects were potentiated by Ang-(1–7) or Ang IV. We conclude that whereas Ang II/AT1R or Ang II/AT2R signaling in the brain stem enhances, Ang-(1–7)/MasR or Ang IV/AT4R antagonizes pressor response after stroke by differential modulations of MCP-1 in the RVLM. Furthermore, combined administration of aliskiren and Ang-(1–7) or Ang IV into the brain stem provides more effective amelioration of stroked-induced pressor response.

Abbreviations: ACE, angiotensin converting enzyme; aCSF, artificial cerebrospinal fluid; Ang II, angiotensin II; Ang IV, angiotensin IV; Ang-(1–7), angiotensin-(1–7); APN, aminopeptidase N; ARBs, angiotensin receptor blockers; AT1R, angiotensin II type I receptor; AT2R, angiotensin II type II receptor; AT4R, angiotensin II type IV receptor; ATRs, angiotensin receptors; BBB, blood–brain barrier; BP, blood pressure; CCL2, chemokine (C–C motif) ligand 2; CCR2, C–C chemokine receptor 2; CSF, cerebrospinal fluid; DBP, diastolic blood pressure; GFAP, glial fibrillary acidic protein; HR, heart rate; i.c.v., intracerebroventricular; ICU, intensive care unit; MasR, Mas receptor; MBP, mean blood pressure; MCAO, middle cerebral artery occlusion; MCP-1, chemokine monocyte chemoattractant protein-1; mNSS, modified neurological severity score; MRI, magnetic resonance imaging; NeuN, neuron specific nuclear protein; RAS, renin–angiotensin system; RVLM, rostral ventrolateral medulla; SBP, systolic blood pressure; TTC, 2,3,5-triphenyltetrazolium chloride.

* Corresponding author at: Center for Translational Research in Biomedical Sciences, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung 833, Taiwan, ROC. Fax: +886 7 7317123x8569.
E-mail addresses: aywchang@mail.ncku.edu.tw, aliceywchang@gmail.com (A.Y.W. Chang).
Available online on ScienceDirect (www.sciencedirect.com).
[1] Present address: Department of Physiology, National Cheng Kung University, Tainan 701, Taiwan, ROC. Fax: +886 6 2362780.

http://dx.doi.org/10.1016/j.nbd.2014.08.005
0969-9961/

Introduction

Stroke is a major global health problem because it is the second leading cause of death worldwide (Donnan et al., 2008). Pressor response after stroke is observed in 60–75% patients (Potter et al., 2005) and is a common complication that leads to poor outcome, including early death in hospital, neurological deficiency at discharge (Zhang et al., 2011) and susceptibility to recurrent stroke (Geeganage et al., 2011). At present, the causes, effects and optimal management of pressor response immediately after stroke remain a hotly debated and sometimes controversial issue (Lattanzi et al., 2013; Sykora et al., 2010). Further delineation of the pathophysiological mechanisms of pressor response after stroke that leads to effective therapeutic management is therefore warranted.

The rostral ventrolateral medulla (RVLM), where sympathetic premotor neurons in the brain stem are located, is responsible for maintaining sympathetic vasomotor tone and stable blood pressure; pharmacological or electrical activation of the RVLM elicits pressor response (Spyer, 1994). Clinically, vascular compression of the RVLM is identified in 26.8% of acute ischemic stroke patients who manifested greater blood pressure variability that is related to poorer prognosis (Aoki et al., 2011). Furthermore, rats in a middle cerebral artery occlusion (MCAO) stroke model (Marks et al., 2001; Mogi et al., 2006) exhibit disturbed cardiovascular responses because of altered neurotransmission in the ventrolateral medulla (Ally et al., 2002). MCAO also augmented Fos-like immunoreactivity in medullary neurons (Wu and Ling, 1998). It follows that the RVLM may participate in pressor response after stroke and is a suitable target for mechanistic delineation.

The condition of up to one-third of stroke patients worsens after hospital admission, and the management of pressor response after stroke is still controversial (Lattanzi et al., 2013; Sykora et al., 2010). Early systemic application of angiotensin receptor blockers (ARBs) reportedly prevents pressor response after stroke (Lüders, 2007) and reduces severity (Lee et al., 2012). Other studies, however, indicate that careful blood pressure lowering treatment with ARBs does not improve the neurological outcomes of patients with stroke (Rhoney and Moser, 2011; Sandset et al., 2011). Whereas angiotensin II (Ang II), the principal effector molecule of the central renin–angiotensin system (RAS), induces tonic sympathoexcitatory and pressor response by acting on Ang II type 1 receptors (AT1R) in the RVLM (Chan et al., 2007, 2010), a decrease of AT1R in the RVLM mediates hypotension induced by lipopolysaccharide administration (Chan et al., 2003). Recent work (Jiang et al., 2012; Faure et al., 2006) further showed that angiotensin-(1–7) (Ang-(1–7)) and angiotensin IV (Ang IV), two metabolites when Ang II is degraded respectively by angiotensin converting enzyme (ACE) 2 or aminopeptidase N (APN) (McKinley et al., 2003), play a protective role during stroke by reducing the infarct volume and subsequent neurological deficits (Faure et al., 2006; Mecca et al., 2011). Considering the importance of the central RAS in blood pressure homeostasis, a systematic evaluation of the roles of Ang-(1–7) and Ang IV and their respective receptor subtypes, Mas receptor (MasR) and AT4R, in addition to Ang II and the classical AT1R and AT2R, in the RVLM in stroke-induced pressor response is therefore of interest.

Monocyte chemoattractant protein-1 (MCP-1), also named chemokine (C–C motif) ligand 2 (CCL2), is a pro-inflammatory chemokine that exhibits potent chemoattractant activity for monocyte/macrophage infiltration to the injured area, of which triggers an intense inflammatory reaction and contributes to worsen stroke brain injury indicated by increased infarct size and impaired neurological outcome (Iadecola and Alexander, 2001). Moreover, MCP-1 contributes to inflammatory reactions during stroke via an action on C–C chemokine receptor 2 (CCR2) (Losy and Zaremba, 2001; Jiang et al., 2008); clinical studies indicated that polymorphism of MCP-1 gene is associated with the susceptibility to stroke (Buraczynska et al., 2010). Elevation of MCP-1 is observed in cerebrospinal fluid (CSF) (Losy and Zaremba, 2001) or in circulation (Arakelyan et al., 2005) of stroke patients and in brain of rats after MCAO (Jiang et al., 2008). In addition, the level of MCP-1 mRNA and MCP-1 concentration in plasma is significantly higher in hypertensive patients (Sardo et al., 2008). Of note is that Ang II induces AT1R-mediated upregulation of MCP-1 or CCR-2, and secretion of MCP-1 in ventricular cardiomyocytes (Omura et al., 2004) or human U937 monocytic cells (Ko et al., 2007). It follows that brain stem MCP-1 may underlie RAS-mediated pressor response after stroke.

Aliskiren, an orally active, non-peptide renin inhibitor, is an antihypertensive agent that blocks renin, the first and rate-limiting enzymatic step of angiotensin synthesis (Sawhney, 2010). Intracerebroventricular (i.c.v.) infusion of aliskiren markedly inhibits the increase in Ang II levels in rat CSF and blood pressure (BP) elicited by i.c.v. infusion of renin (Huang et al., 2012). Furthermore, pretreatment with systemic application of aliskiren reduces the expression of inflammatory marker genes in the cerebral ischemic core after MCAO (Schmerbach et al., 2010). Because of the controversy surrounding the efficacy of ARBs against pressor response after stroke, whether aliskiren-mediated reduction of inflammatory response presents itself as an alternative therapeutic strategy warrants further delineation.

Employing a MCAO stroke model, the present study was undertaken to assess the hypothesis that Ang II, Ang-(1–7) or Ang IV, and AT1R, AT2R, MasR or AT4R, contribute to pressor response after stroke by differential modulations of pro-inflammatory MCP-1 in the RVLM. We also investigated the relationship between these cellular mechanisms and the beneficial effects of aliskiren.

Materials and methods

All experimental procedures carried out in this study have been performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, approved by the Institutional Animal Care and Use Committee of the Kaohsiung Chang Gung Memorial Hospital, and were in compliance with the guidelines for care and handling of animals set forth by that Committee.

Animals

Adult male Sprague–Dawley rats (285–337 g, n = 219) purchased from the Experimental Animal Center of the National Science Council, Taiwan, Republic of China were used. Animals were housed in groups of 2–3 in individually ventilated cages in an AAALAC International-accredited Center for Laboratory Animals, with free access to rat chow and water. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Blood pressure and heart rate measurement

Systolic and diastolic blood pressure (SBP and DBP), mean BP (MBP) and heart rate (HR) of conscious rats were measured between 0900 and 1200 h, using a non-invasive tail cuff plethysmography (Visitech Systems, Apex, NC, USA). Averages of 10 inflation/deflation cycles were conducted to obtain MBP.

MCAO stroke model

Transient MCAO was induced by intraluminal vascular occlusion as described previously (Marks et al., 2001; Mogi et al., 2006; Ally et al., 2002; Wu and Ling, 1998). Briefly, the left MCA in rats anesthetized with 2–3% isoflurane was effectively occluded by a silicon-coated 4-0-monofilament (Doccol, Sharon, MA, USA) for 120 min, followed by reperfusion. Successful establishment of experimental stroke was confirmed on day 1 after MCAO by magnetic resonance imaging (MRI) and staining with 2,3,5-triphenyltetrazolium chloride (TTC; Sigma). Specifically, the presence of edema was determined by high resolution T2 images obtained from a 9.4 T Animal MR scanner (Biospec 94/20, Bruker, Ettlingen, Germany). For TTC staining, rats deeply anesthetized with pentobarbital sodium were perfused intracardially with warm saline that contains