Neuroscience Letters 661 (2017) 126–131

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

Transgenic expression of HuR increases vasogenic edema and impedes functional recovery in rodent ischemic stroke

Agnieszka A. Ardelt[a,*,1], Randall S. Carpenter[a,1], Ifeanyi Iwuchukwu[b], An Zhang[a], William Lin[a], Ewa Kosciuczuk[c], Cyrus Hinkson[a], Tania Rebeiz[a], Sydney Reitz[a], Peter H. King[d]

[a] Department of Neurology, University of Chicago, 5841 S. Maryland Ave, MC2030, Chicago, IL 60637, United States
[b] Department of Neurocritical Care, Ochsner Medical Center, 1514 Jefferson Hwy., New Orleans, LA 70121, United States
[c] Division of Hematology-Oncology, Northwestern University, 675 North St. Clair, Chicago, IL 60611, United States
[d] Department of Neurology, University of Alabama at Birmingham, 1720 7th Avenue South, Birmingham, AL 35233, United States

ARTICLE INFO

Keywords:
Ischemic stroke
Transgenic mice
Brain edema
Middle cerebral artery occlusion
Vascular permeability

ABSTRACT

Background and purpose: Ischemic stroke produces significant morbidity and mortality, and acute interventions are limited by short therapeutic windows. Novel approaches to neuroprotection and neurorepair are necessary. HuR is an RNA-binding protein (RBP) which modulates RNA stability and translational efficiency of genes linked to ischemic stroke injury.

Methods: Using a transgenic (Tg) mouse model, we examined the impact of ectopic HuR expression in astrocytes on acute injury evolution after transient middle cerebral artery occlusion (tMCAO).

Results: HuR transgene expression was detected in astrocytes in perilesional regions and contralaterally. HuR Tg mice did not improve neurologically 72 h after injury, whereas littermate controls did. In Tg mice, increased cerebral vascular permeability and edema were observed. Infarct volume was not affected by the presence of the transgene.

Conclusions: Ectopic expression of HuR in astrocytes worsens outcome after transient ischemic stroke in mice in part by increasing vasogenic cerebral edema. These findings suggest that HuR could be a therapeutic target in cerebral ischemia/reperfusion.

1. Introduction

Ischemic stroke is a significant cause of morbidity and mortality world-wide, and only a fraction of acute stroke patients is eligible for thrombolysis or thrombectomy [15]. Despite promising preclinical results, there are no proven neuroprotective agents for acute ischemic stroke [34]. This clinical problem provides the rationale for identifying acutely activated molecular pathways which modulate secondary tissue injury or neural repair.

We investigated post-transcriptional gene regulation as a novel pathway in cerebral ischemia/reperfusion. Genes linked to secondary tissue injury including pro-inflammatory cytokines (e.g., IL-1β and TNF-α) and matrix metalloproteinases (e.g., MMP-9 and 12) increase in the hyperacute phase and are modulated post-transcriptionally [3,10,11,13,16,21,26,28,32]. The key elements in post-transcriptional gene regulation are adenine- and uridine-rich elements (AREs) in the 3′ and/or 5′ untranslated regions of mRNA [1,3,7]. AREs, through interaction with RNA-binding proteins (RBPs), modulate mRNA stability and translational efficiency. RBPs such as KH-type splicing regulatory protein (KSRP) and tristetraprolin (TTP) are negative regulators, whereas HuR is generally a positive regulator. RBPs can dramatically affect target mRNA and protein levels. In astrocytes, knockdown of HuR led to a 30–40% attenuation of factors relevant to ischemia such as TNF-α, IL-1β, and MMP-12, whereas knockout of KSRP led to significant increases [13,20,21,24,26]. These observations provided the basis for our hypothesis that HuR affects secondary tissue injury in experimental ischemic stroke. We used a transgenic (Tg) mouse model in which human HuR is selectively expressed in astrocytes using the glial fibrillary acidic protein (GFAP) promoter [37]. After exposure to transient cerebral ischemia, we observed increases in perilesional HuR transgene expression, vascular permeability, and cerebral edema. Tg mice exhibited worse short-term neurologic outcomes. These findings highlight the

* Corresponding author.
E-mail addresses: aardelt@yahoo.com (A.A. Ardelt), carpenter.794@buckeyemail.osu.edu (R.S. Carpenter), iiwuchukwu@gmail.com (I. Iwuchukwu), anzhang518@gmail.com (A. Zhang), william.lin.phd@gmail.com (W. Lin), ewa.kosciuczuk@northwestern.edu (E. Kosciuczuk), clhinkson223@gmail.com (C. Hinkson), Tania.Rebeiz@uchospitals.edu (T. Rebeiz), sydneyreitz@gmail.com (S. Reitz), phking@uabmc.edu (P.H. King).
[1] Equal contribution.

http://dx.doi.org/10.1016/j.neulet.2017.09.062
Received 2 August 2017; Received in revised form 25 September 2017; Accepted 29 September 2017
Available online 02 October 2017

importance of post-transcriptional regulation in ischemic stroke and identify HuR as a potential therapeutic target.

2. Materials and methods

2.1. Animals

Procedures were approved by the University of Chicago Institutional Animal Care and Use Committee and were in accordance with NIH guidelines for the use of animals in research. The Tg mouse line was previously described [37]: the GFAP promoter was utilized to direct expression of the human HuR gene coupled with the FLAG octapeptide tag in C57Bl/6J mice. Animals were housed in cages with littermates on a 6 AM–6 PM light cycle. Mice were fed Teklad Diet 2918 (Harlan Laboratories, Madison, WI) ad libitum.

2.2. Blinding, allocation, group size, and endpoints

Technicians and investigators were blinded to the genetic background of the mice. Wild-type (Wt) and Tg mice are externally indistinguishable. Mice were randomly tagged with sequentially numbered ear tags at weaning and underwent experimental ischemic stroke when 8–10 weeks old. Male mice are reported in this manuscript; female mice were also studied but are not reported here. Animals housed in each cage (between three to six mice) underwent transient middle cerebral artery occlusion (tMCAO) on the same day. All cages housed Wt with Tg mice. There were four cohorts of animals: Wt, 24-h survival (final n = 20); Tg, 24-h survival (final n = 20); Wt, 72-h survival (final n = 26); and Tg, 72-h survival (final n = 23). Target cohort size of 20 was calculated based on a 15% difference in infarct volume 24 h after tMCAO in Wt mice. Target cohort size was inflated by 20% to account for expected increased mortality by 72 h.

2.3. tMCAO, sham tMCAO, and neurologic score

Mice were anesthetized with isoflurane (induction: 5%; maintenance: 1.5–2%) in oxygen (0.05 L/min) and medical air (1 L/min). After sterile preparation and draping, tMCAO was performed as previously described [5] with minor modifications. Briefly, the right common carotid artery (CCA) was exposed, and a 7–0 nylon suture tipped with dental adhesive was introduced into the CCA until laser Doppler signal (Periflux System 5000, Perimed, Inc., Ardmore, PA) from a probe positioned over the MCA territory dropped by ≥80%. During MCAO, rectal temperature was maintained between 36.5 and 37.5 °C with a homeothermic system (Harvard Apparatus, Holliston, MA). After 30 min, the occluding thread was removed, and the neck was sutured. One hour after reperfusion, the neurologic score was determined: 0 = no deficit; 1 = twists when suspended by the tail; 1.5 = veers while walking; 2 = circles while walking; 3 = rolls; 4 = no movement. Euthanasia was performed 24 or 72 h later. For sham tMCAO, the occluding thread was not advanced into the MCA.

2.4. Immunolabeling

8 μm-thick frozen sections at bregma 0.86 were fixed in 4% paraformaldehyde and labeled with anti-FLAG (F7425, Sigma-Aldrich Corp., St. Louis, MO) and anti-GFAP (ab5541, EMD Millipore, Billerica, MA) antibodies, per manufacturers' protocols. Cy3-conjugated (AP194C, Millipore) and Alexa Fluor 488-conjugated (A11008, Life Technologies, Grand Island, NY) secondary antibodies were used. Regions of interest (ROI), were digitally imaged (10× objective; BX41 microscope and DP72 digital camera, Olympus America Inc., Lombard, IL; Data in Brief) and FLAG-positive cells were counted per ROI and averaged for three tissue sections/animal.

Additional 8 μm-thick sections were fixed with 10% buffered formalin and labeled with an anti-GFAP antibody (Z0334, Dako, Carpinteria, CA), 1:5,000 followed by Alexa Fluor 488-conjugated secondary antibodies (A11001, Life Technologies). Sections were then counterstained with Hoechst 33258 (Sigma) and double-labeled for MAP2 (ab5392, Abcam, Cambridge, MA) followed by a Cy3-conjugated secondary (AP194C, Millipore).

Additional 8 μm-thick sections were fixed with 4% paraformaldehyde and immunolabeled with anti-FLAG and anti-MAP2 (ab5392, Abcam) antibodies followed by the secondary antibodies described above.

2.5. Infarct volume and swelling

Additional 8 μm-thick frozen sections from mouse brains at bregma levels 1.10, 0.86, 0.50, −0.70, −1.22, −1.94, −2.54, and −3.64 were stained with hematoxylin and eosin (HE) and digitally imaged (1.25× objective plus in-line 0.5 × video adapter, Olympus). Metamorph Offline, v7.7 (Molecular Devices Corp., Sunnyvale, CA) was utilized to outline remnant (normal neuropil) ipsilateral areas and contralateral and ipsilateral hemispheres. Lesional volumes were calculated in mm^3 and expressed as a percentage: (contralateral hemisphere volume − ipsilateral remnant volume)/contralateral hemisphere volume * 100%. Hemispheric swelling was calculated as: (ipsilateral hemisphere volume − contralateral hemisphere volume)/contralateral hemisphere volume * 100%.

2.6. Evans Blue content

Mice were anesthetized, and 4 μl/g of filter-sterilized 2% Evans Blue in normal saline was injected intravenously. Mice were euthanized two hours later; 25 ml of cold (4 °C) phosphate-buffered saline (PBS) was systemically perfused via cardiac puncture. Brains were dissected into left and right hemispheres, and the olfactory bulbs and cerebella were removed. Hemispheres were homogenized in cold PBS (Omni Tissue Homogenizer, Omni International, Kennesaw, GA) and extracted with trichloroacetic acid (Sigma). Supernatants were assayed at 620 nm excitation/690 nm emission (Tecan Safire 2, Tecan Group Ltd., Männedorf, Switzerland).

2.7. qRT-PCR

Ten 8 μm-thick frozen sections per mouse brain were collected at bregma levels 1.10 to −1.94. Infarcts were visible to the naked eye and infarcted tissue was scraped off the slides with a sterile microblade (#37-6100, Roboz Surgical Instrument Co., Inc., Gaithersburg, MD) and homogenized in 600 μl RLT lysis buffer (#74104, Qiagen, Boston, MA). Symmetrical contralateral (non-infarcted) tissue was similarly processed. Total RNA was isolated from homogenates using RNeasy Mini Kit (#74104, Qiagen,) per manufacturer's protocol. 100 ng of total RNA per sample was reverse-transcribed into cDNA using Quantifect Reverse Transcription Kit (#205311, Qiagen). The cDNA was used for qRT-PCR using fluorogenic 5′-nuclease assay technology with probes and primers for the housekeeping gene peptidylprolyl isomerase A (PPIA; Integrated DNA Technologies, Coralville, IA) and TaqMan® Gene Expression Assay Reagents (#4331182, Life Technologies, Carlsbad, CA) for mouse VEGFa. Individual gene amplification was performed in duplicate with the CFX96 Touch Real-time PCR Detection System (185-5096, Biorad, Des Plaines, IL, USA) using Universal PCR Master Mix AmpliTaq Gold DNA Polymerase with Buffer II and MgCl2 (N8080247_3620536626, Life Technologies). The threshold cycle, $C_{T,}$ was determined using CFX Manager™ software v3.1 (Biorad).

2.8. Statistical analysis

Data are presented as mean ± SD except where otherwise indicated. Student's *t*-test, Mann-Whitney rank sum test, Wilcoxon signed rank sum test, or Fisher's exact test were used.