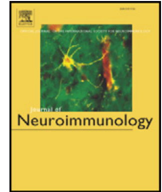
Journal of Neuroimmunology 287 (2015) 36–42

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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



Gelsolin decreases actin toxicity and inflammation in murine multiple sclerosis

Kevin Li-ChunHsieh [a,1], Stefan Schob [a], Matthias W.G. Zeller [a], Benjamin Pulli [a], Muhammad Ali [a], Cuihua Wang [a], Terry Ting-Yu Chiou [b], Yuk-Ming Tsang [c], Po-Shun Lee [b], Thomas P. Stossel [b], John W. Chen [a,*]

[a] Center for Systems Biology, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, MA, USA
[b] Translational Medicine Division, Brigham & Women's Hospital, Harvard Medical School, MA, USA
[c] Division of Medical Imaging, Radiology Department, Far Eastern Memorial Hospital, New Taipei City, Taiwan

ARTICLE INFO

Article history:
Received 23 March 2015
Received in revised form 26 July 2015
Accepted 4 August 2015

Keywords:
Gelsolin
Experimental autoimmune encephalomyelitis (EAE)
Actin
Inflammation
Myeloperoxidase
Magnetic resonance imaging

ABSTRACT

Gelsolin is the fourth most abundant protein in the body and its depletion in the blood has been found in multiple sclerosis (MS) patients. How gelsolin affects the MS brain has not been studied. We found that while the secreted form of gelsolin (pGSN) decreased in the blood of experimental autoimmune encephalomyelitis (EAE) mice, pGSN concentration increased in the EAE brain. Recombinant human pGSN (rhp-GSN) decreased extracellular actin and myeloperoxidase activity in the brain, resulting in reduced disease activity and less severe clinical disease, suggesting that gelsolin could be a potential therapeutic target for MS.
© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Multiple sclerosis (MS) is the leading cause of non-traumatic neurological disability in young adults (Noseworthy et al., 2000). While the etiology of MS remains unknown, it is characterized by inflammatory demyelinating plaques in the CNS (Bruck, 2005; Frohman et al., 2006; Imitola et al., 2006; Noseworthy et al., 2000). A multitude of inflammatory factors has been investigated in MS, but relatively little is known about whether gelsolin (GSN), the fourth most abundant protein in the human body (Smith et al., 1987), has a role in MS.

Gelsolin is the first and most widely expressed member of a family of actin-severing proteins in humans (Yin and Stossel, 1979). Gelsolin is not only mainly produced from the muscle tissue but also expressed in the human central nervous system (Kwiatkowski et al., 1988; Tanaka et al., 1993). It is involved in actin homeostasis, cell exocytosis, cell motility, phagocytosis, apoptosis, platelet formation and activation (Lee and Galbraith, 1992; Osborn et al., 2007; Silacci et al., 2004). It has intracellular and secreted (termed pGSN) forms in the human body, which are structurally similar but not identical (Yin et al., 1984). Both forms are from the same gene on the 9th chromosome, but the secreted form differs from the intracellular form by a 25-amino acid signaling peptide and the presence of a disulfide bond between cysteine residues at positions 188 and 201 (Wen et al., 1996; Yin et al., 1984).

While a decrease of total gelsolin level in the blood was found in multiple sclerosis patients (Kulakowska et al., 2010), it is yet unknown how multiple sclerosis affects secreted gelsolin in the brain (Yin et al., 1984), and whether modulating gelsolin levels could have a therapeutic benefit for multiple sclerosis. Therefore, we aimed to evaluate the effects of neuroinflammation on the levels of pGSN in the brain in a mouse model of multiple sclerosis (experimental autoimmune encephalomyelitis [EAE]) and to determine whether gelsolin has a therapeutic role for EAE/MS.

2. Materials and methods

2.1. EAE induction

All experimental protocols were approved by the Institutional Animal Care and use Committee of Massachusetts General Hospital. 111 SJL female mice 6–10 weeks old (Jackson Laboratories, Bar Harbor,

Abbreviations: EAE, experimental autoimmune encephalomyelitis; MPO, myeloperoxidase; GSN, gelsolin; pGSN, plasma gelsolin; rhp, recombinant human plasma; rhp-GSN, recombinant human plasma gelsolin.

* Corresponding author at: 185 Cambridge Street, Boston, MA 02114, USA.
E-mail address: jwchen@mgh.harvard.edu (J.W. Chen).
[1] Present address: Department of Medical Imaging, Taipei Medical University Hospital, Taipei, Taiwan and Translational Imaging Research Center, College of Medicine, Taipei Medical University, Taipei, Taiwan.

http://dx.doi.org/10.1016/j.jneuroim.2015.08.006
0165-5728/© 2015 Elsevier B.V. All rights reserved.

ME) were used for this study. EAE was induced with synthetic proteolipid protein (PLP139-151, Axxora, CA) according to Greer et al. (1996). Briefly, 2 mg of PLP and 8 mg *Mycobacterium tuberculosis* H37Ra (Difco, MI) in 1 ml water and 1 ml of complete Freund's adjuvant (Sigma-Aldrich, MO) were emulsified. Each mouse received 100 μl of the emulsion (25 μl each in the bilateral inguinal and axillary regions). On days 0 and 2, 0.1 mg of Pertussis toxin was injected intravenously via the tail vein. The animals were monitored at least daily with the following clinical grading: 0 = normal, 1 = tail limpness, 2 = hind limb weakness, 3 = complete hind limb paralysis, 4 = unresponsive and moribund, and 5 = found dead in cage. 15 mice died shortly after EAE induction and were removed from the study. Sham-induced mice (n = 6) were induced with all of the above except for PLP which was not administered.

2.2. Blood and brain specimen preparation

Retro-orbital blood was centrifuged at 2500 rpm for 15 min at 4 °C. The supernatant was collected and mixed with 1× RIPA lysis buffer (Millipore, MA) in PBS v/v for 2 h on ice and then centrifuged at 14,000 rpm for 20 min. The supernatant was used for protein quantification with the bicinchoninic acid kit (Thermo Fisher Scientific, MA) and for Western blot of pGSN.

Mouse brains were homogenized and proteins were extracted in 1× RIPA lysis buffer (Millipore, MA) in PBS v/v. The resultant suspension was sonicated for 15 s and then incubated on ice for 2 h. The suspensions were centrifuged at 14,000 rpm for 20 min, and used for protein quantification and Western blot of pGSN.

2.3. Western blot

25 g of protein from plasma or brain was loaded per well and samples were separated using Tris–HCl Gels (Bio Rad, CA) and then transferred onto PVDF membranes (IMMUNO-Blot PVDF Membrane, Bio Rad) using a wet tank blotting system (BioRad). Membranes were blocked in 5% milk at 4 °C overnight and then incubated with a rabbit polyclonal antibody directed against plasma extension of mouse gelsolin at a dilution of 1:500 in 5% milk (Chou et al., 2011). This antibody does not recognize cytoplasmic gelsolin. To detect actin, membranes were incubated with 1:200 dilution of a rabbit polyclonal anti-actin antibody (Sigma-Aldrich, MO, #A2066). β-Tubulin (rabbit polyclonal antibody at 1:500, Abcam, MA, #ab4074) and albumin (rabbit polyclonal antibody at 1:500, Abgent, CA, #ABIN390450) were used as loading controls in brain and serum samples, respectively. We used a chemiluminescence system and peroxidase-conjugated secondary antibody for detection of protein bands.

2.4. rhp-GSN supplement therapy

Recombinant human plasma (rhp)-GSN was produced in *Escherichia coli*, refolded with oxidized glutathione, purified (Lee et al., 2007), and brought to a final stock protein concentration of 10 mg/ml in PBS (vehicle). For each mouse, 200 mg/kg rhp-GSN split into two doses on either days 0 and 2 or days 8 and 10 of EAE induction was administered intravenously. Gelsolin has a blood half-life of about 2.3 days (Smith et al., 1987) and was expected to remain elevated up to 7–9 days after administration.

2.5. Extracellular protein extraction

The protocol of extracellular protein extraction was adopted from Hofstein et al. (1983) and Pulli et al. (2013). After cardiac perfusion with 20 ml cold saline in an anesthetized mouse, brains were harvested and washed in PBS three times. We then incubated organs in 4 times their weight HBSS with 0.32 M sucrose, 1 mM calcium acetate, 10 U/ml heparin, and proteinase inhibitor on ice for 2 h. After this procedure, we added four parts ice-cold acetone per part buffer to the samples and incubated for 1 h at −20 °C to precipitate proteins. Samples were then centrifuged at 4000 *g* for 20 min. The pellets were dried for 1–3 min and resuspended in 0.2 mL of PBS and stored for further analyses.

2.6. Antibody-captured MPO activity assay

Antibody-captured MPO activity assay was performed according to Pulli et al. (2013). 100 μl of the 1:10 diluted samples was added per well into the MPO ELISA plates (Hycult Biotech, PA), incubated for 1 h at 4 °C. After washing five times with washing buffer, we added 50 μl of 200 μM Amplite ADHP (AAT Bio, prepared in DMSO), 49 μl PBS and 1 μl 0.03% H_2O_2 working solution into each well. We then acquired fluorescence using a plate reader for 5–10 min (10–50 cycles) with excitation = 535 nm, and emission = 590 nm. The units of activity were computed according to the following formula: Activity = (ΔOD × Vt × 4) / (E × Δt × Vs), where ΔOD = change in absorbance; Vt = total volume; Vs = sample volume; E (extinction coefficient) = 26.6 mM^{-1}; and Δt = change in time.

2.7. MPO-Gd magnetic resonance imaging

To confirm the presence of myeloperoxidase (MPO) activity within inflammatory brain plaques in vivo and to evaluate the anti-inflammatory effect of rhp-GSN non-invasively in living animals, we performed MPO-Gd (bis-5-hydroxytryptamide-diethylenetriaminepentaacetate-gadolinium) molecular MRI on day 11 after induction, at the time of early acute disease onset, with and without rhp-GSN therapy (n = 4 per group). MPO-Gd is an activatable MR imaging agent that reports extracellular MPO activity in vivo with high specificity and sensitivity (Breckwoldt et al., 2008; Chen et al., 2008). MR imaging was performed by using an animal 4.7-T MR scanner with a dedicated head coil (Bruker Instruments, Billerica, MA). Mouse axial T1-weighted (RARE sequence, TR = 873, ms, TE = 8.48 ms, fourteen averages, matrix size 192 × 192, field of view 2.5 × 2.5 cm^2, slice thickness 0.7 mm, and 16 sections were acquired) images were obtained before and after intravenous administration of MPO-Gd (0.3 mmol/kg). Post-contrast images were acquired for at least 60 min.

MR images were independently evaluated by two independent authors blinded to the treatment groups using OsiriX. Contrast-to-noise ratios (CNR) were computed for each region of interest (ROI) according to the formula: CNR = (ROI lesion − ROI normal brain) / SD noise, where ROI lesion is the ROI of an enhancing lesion, ROI normal brain indicates the ROI of an unaffected area of the brain, and SD noise is the standard deviation of noise from an ROI measuring empty. Comparisons of CNR, lesion number, lesion size and total lesion area were performed by visually counting the number of and area of enhanced lesions over the entire brain for each mouse, and the results were averaged.

2.8. Flow cytometry

Mice were transcardially perfused with ice-cold PBS, brains were harvested and leukocytes were isolated by density centrifugation. Brains were mechanically dissociated in 7 ml of 30% Percoll using a douncer homogenizer and loose fitting pestle. The suspension was then filtered through a 40 μm cell strainer (BD Biosciences, San Jose, CA), underlayed with 3 ml of 70% Percoll and overlayed with 2 ml DPBS. The gradient was then centrifuged at 650 *g*, 18 °C for 25 min. After centrifugation, the thick myelin layer at the 0/30 interface was discarded and the brain leucocytes at the 30/70 interface were collected. The cells were then stained for flow cytometry. All antibodies were purchased from BD Biosciences unless otherwise noted. Antibodies used were anti-CD90.2 (clone: 53-2.1) ; anti-NK1.1 (clone: PK136, eBioscience); anti-CD49b (clone: DX5); anti-Ly-6G, (clone: IA8); anti-TER119 (clone: TER119, eBioscience), anti-CD3 (clone: 17A2) and

Download English Version:

https://daneshyari.com/en/article/3063901

Download Persian Version:

https://daneshyari.com/article/3063901

Daneshyari.com