Int J Clin Exp Med 2016;9(12):23102-23110
www.ijcem.com /ISSN:1940-5901/IJCEM0020247

Original Article

Effect of interferon-β on the expression of cytoskeleton and cellular elasticity associated protein in astrocytes

Xiaobo Yan, Chenye Li, Shuang Wang, O Lv, Mingwen Xuan

Department of Neurology, The Second Clinical Hospital of Harbin Medical University, Harbin 150086, China

Received November 21, 2015; Accepted February 10, 2016; Epub December 15, 2016; Published December 30, 2016

Abstract: Objective: To investigate the effect of interferon β (IFN-β) on the cytoskeleton, cellular elasticity and the expressions of cytoskeleton and cellular elasticity associated protein in cultured astrocytes. *Methods:* The astrocytes were treated with IFN-β (10^2 and 10^3 U/mL) for 24, 48 and 72 h. The shape and elasticity of astrocytes were observed by atomic force microscope. The ultrastructural of astrocytes was observed by scanning electron microscopy and transmission electron microscope. The cell viability was measured by MTT method. The expression of cytoskeleton-elasticity associated protein, including glial fibrillary acidic protein (GFAP), β-actin, cofilin-1, profilin-1, and vimentin were detected by Western Blot. Result: Compared with the control group, the structure of intracellular microfilaments was more obvious, the cell junctions were obviously increased and tighter, the cells elastic modulus was higher and cytoskeletons were obviously increased after treatment with 10^2 and 10^3 U/mL IFN-β for 72 h. Treatment with IFN-β (10^2 and 10^3 U/mL) for 24, 48 and 72 h did not affect the growth of AS. Compared with the control group, the expressions of cofilin-1, GFAP, profilin-1 and vimentin in AS were significantly increased in treatment with 10^3 U/mL IFN-β for 48 h and 10^2 and 10^3 U/mL IFN-β for 72 h group (all $P < 0.05$). *Conclusion:* IFN-β increases the flexibility and toughness of AS through up-regulation the expressions of cytoskeleton-elasticity associated protein cofilin-1, GFAP, profilin-1 and vimentin.

Keywords: IFN-β, astrocytes, cytoskeleton, cell elasticity, multiple sclerosis

Introduction

Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS) mediated by T cells. Its clinical manifestations include progressive loss of nervous system function. Young people are prone to develop this disease. The clinical manifestation is various and the course is long. The multiple sites may be involved and eventually the character of severe disability will occur in MS [1, 2]. The research of MS has been focused on the effector T cells with little research about intrinsic cells. The astrocytes (AS), as the most content of the CNS, played an important role in the pathogenesis of MS. Studies have shown that the effector cells must go through the blood-brain barrier (BBB) into the CNS before they take effect. As an inherent part of the BBB, the activation of AS mediates cell signaling and cell antigen-presenting, thus affecting cell migration. However, during the process of MS, AS hyperplasia, glial plaque formation also can aggravate the condition of MS [3, 4]. Thus it can be seen that the regulation and morphology of astrocytes plays an important role in the pathogenesis of MS. Studies have demonstrated that interferon α (IFN-α) suppressed the pathological plaque formation [5]. However, there are little studies about the role of interferon β (IFN-β) which is a commonly used drug in the regulation of CNS glial plaque formation.

IFN-β is a kind of glycoprotein produced by fibroblasts after stimulation by inflammation or virus-induced factors. IFN-β not only inhibits the activation and proliferation of T cells, also promotes the apoptosis of autoreactive T cells and generation of regulatory T cells, which prevents the leukocytes from going across the BBB. IFN-β adjusts the astrocytes cell morphology to strengthen the selective permeability through the BBB, then plays a treatment effect to MS [6, 7]. However, some patients appeared a reduced sensitivity to IFN-β after long-term use of IFN-β, even sicker illness state [8].

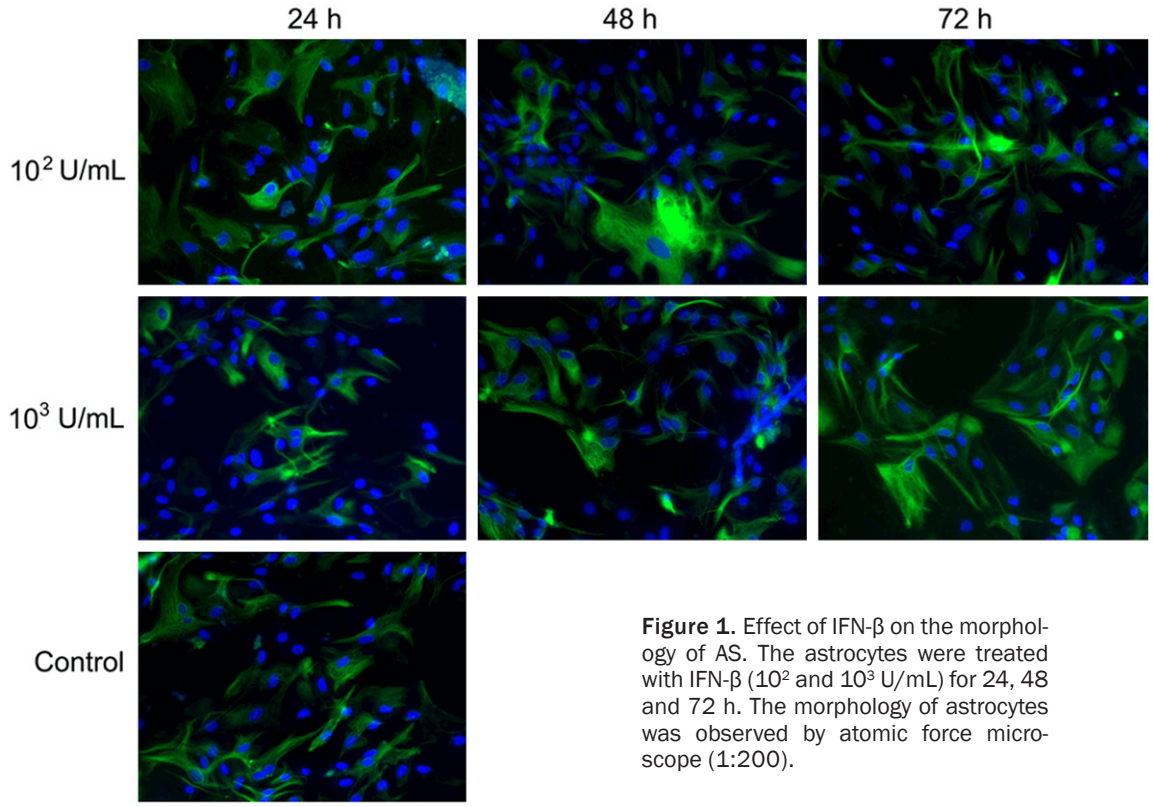

Figure 1. Effect of IFN-β on the morphology of AS. The astrocytes were treated with IFN-β (10^2 and 10^3 U/mL) for 24, 48 and 72 h. The morphology of astrocytes was observed by atomic force microscope (1:200).

Therefore, our research was aimed to investigate the effect of IFN-β on the cytoskeleton, cellular elasticity and the expressions of cytoskeleton and cellular elasticity associated protein in cultured astrocytes and to explore a new target for MS treatment.

Materials and methods

Primary culture of astrocytes and experimental groups

The newborn Sprague-Dawley (S-D) rats (24 to 48 h) were selected. S-D rats were provided by the Experimental Animal Center of Harbin Medical University. The rats were sacrificed by cervical dislocation. The cerebral cortex organizations of rats were shredded, digested, filtrated and centrifuged to prepare signal cell suspension. The cells were cultured at 37°C in 5% CO_2 and 95% air. The oscillation and purification was processed at the 10th day. The rate of positive cells was 90% with glial fiber acidic protein (GFAP) immunofluorescence check. The AS was divided into control group and IFN-β treatment groups (treatment with 10^2 and 10^3 U/mL IFN-β for 24, 48 and 72 h).

Atomic force microscope assay

Astrocytes were climbed the film. After the cell-attached covered coverslip, the cells were treated with 10^2 and 10^3 U/mL IFN-β for 24, 48 and 72 h. The cells were fixed with glutaraldehyde for 1 h. The changes of cell shape and elasticity was observed and photographed under atomic force microscopy. The elastic modulus was calculated.

Scanning electron microscopy

After the cell-attached covered coverslip, the cells were treated with 10^2 and 10^3 U/mL IFN-β for 24, 48 and 72 h. At the different action time points, the cells were washed with PBS for three times of 5 min and fixed with glutaraldehyde for 1 h. The morphological changes were further observed and photographed under the scanning electron microscopy.

Transmission electron microscope (TEM)

The cells were passaged and seeded in culture flask. When the cell growth of this batch cultured cells in the flasks was substantially same, the culture flasks were grouped and the cells

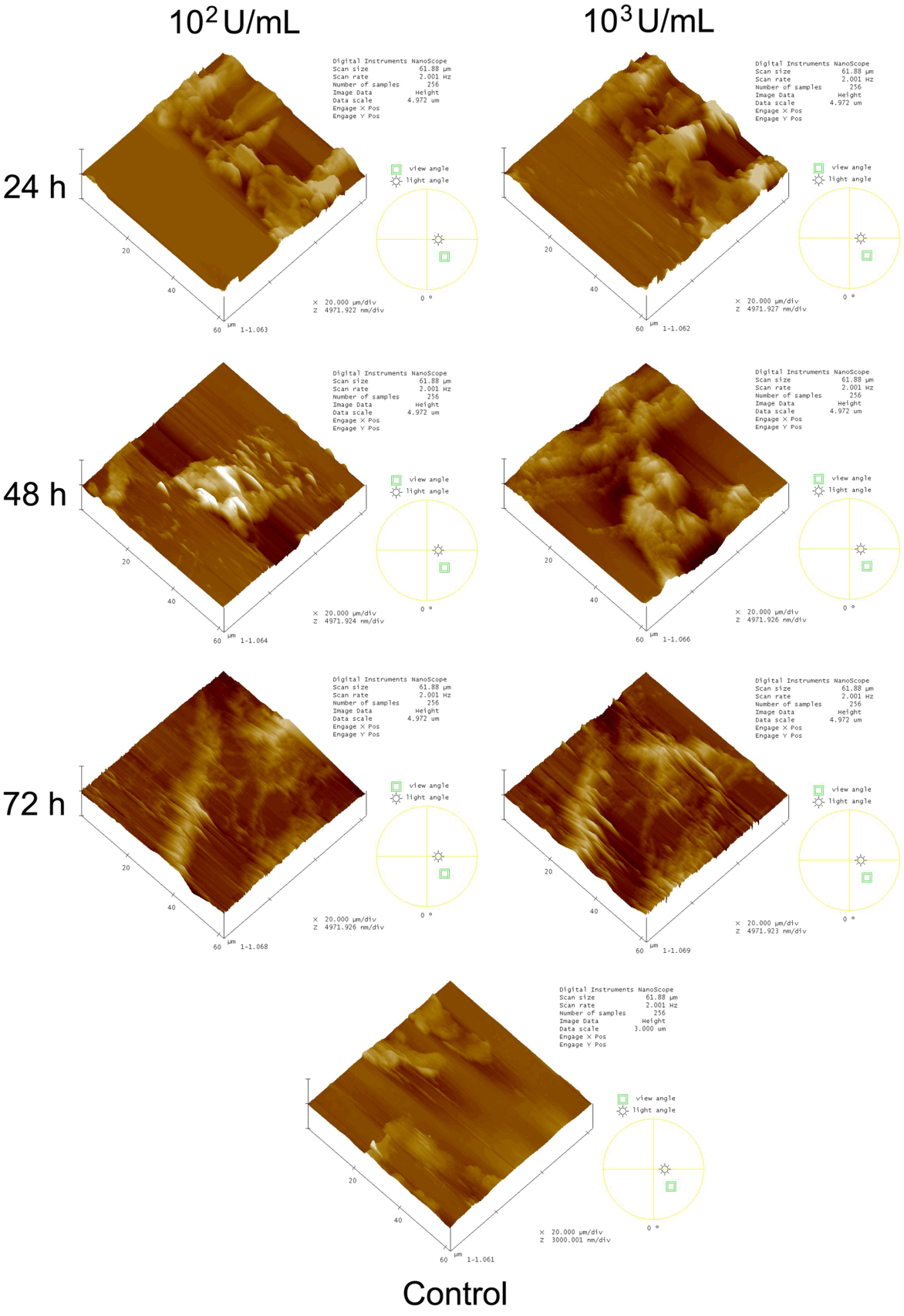
10^2 U/mL
10^3 U/mL
24 h
48 h
72 h
Control
Digital Instruments NanoScope
Scan size 61.88 µm
Scan rate 2.001 Hz
Number of samples 256
Image Data Height
Data scale 4.972 um
Engage X Pos
Engage Y Pos
view angle
light angle
20
40
60 µm
1-1.063
1-1.062
1-1.064
1-1.066
1-1.068
1-1.069
1-1.061
× 20.000 µm/div
Z 4971.922 nm/div
Z 4971.927 nm/div
Z 4971.924 nm/div
Z 4971.926 nm/div
Z 4971.923 nm/div
Z 3000.001 nm/div
0 °

Figure 2. The cell morphology observed by atomic force microscopy (4.972 μm).

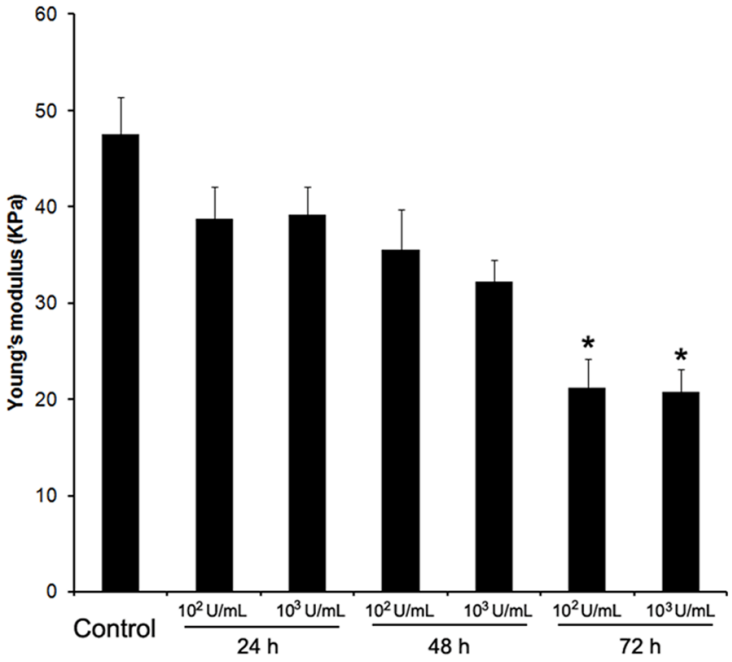

Figure 3. Effect of IFN-β on the value of cell elasticity of astrocyte. The astrocytes were treated with IFN-β (10^2 and 10^3 U/mL) for 24, 48 and 72 h. Histograms showed the value of Young's modulus. Data were expressed as mean ± S.D (n = 3). *P < 0.05 compared with the control group.

were treated with 10^2 and 10^3 U/mL IFN-β for 24, 48 and 72 h. At the different action time points, AS in each group were respectively scraped off with cell scraper and collected in the EP tube. AS were fixed with glutaraldehyde for 1 h. The structures of organelles and cell junctions were observed under TEM.

MTT assay

AS were observed under a microscope. The state of the cell growth was good without population. The cells were digested with trypsin. The cell suspension was made with DMEM medium containing 10% FBS. 5×10^3 per well were seeded in 96-well plates. Each well included 200 μL of culture solution. The plates were shifted into CO_2 incubator with 5% CO_2, 37°C. After the adherent growth of these cells, different concentration of IFN-β was added in the culture medium. The blank control group, control group, 10^2 U/mL group and 10^3 U/mL group was set respectively. Each group had the different acting time points at 24, 48 and 72 h. Then, each well was added the 20 μL MTT solution (5 mg/mL). After 4 h continued culture, the supernatant was discarded and each well was added with 150 μL DMSO. After oscillation, the absorbance value at 492 nm wavelength of each well was measured on a microplate reader. The horizontal axis of the cell growth curve stand for concentration and time point, and the longitudinal axis stand for the absorbance value.

Western blot assay

The cells were homogenized in ice-cold buffer containing 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% SDS, 1% sodium deoxycholate, 50 μL/g protease inhibitor cocktail and 1% Triton X-100. Cells homogenates were centrifuged at 12,000 g at 4°C for 15 min. Protein concentration of the supernatants was determined by using BCA Protein assay Kit (Rockford, IL, USA). Then the protein samples were subjected to SDS-PAGE. Following electrophoresis, the proteins were transferred electrophoretically to PVDF membranes (Rockford, IL, USA). The PVDF membranes were blocked with 3% fat-free milk for 1 h at 37°C. Then the membranes were incubated with primary antibody (Santa Cruz, CA, USA) of actin-binding protein (cofilin-1), glial fibrillary acidic protein (GFAP), profilin-1, vimentin and β-actin diluted in 0.1% BSA (dilution at 1:200) at the room temperature overnight. After the membranes were washed three times with Tris-buffered saline, they were incubated with horseradish peroxidase-conjugated secondary antibody for 2 h at the room temperature. Immunoreactivity was detected by enhanced chemiluminescence (ECL). The band density was measured with Quantity One analysis software (Bio-Rad, Hercules, CA, USA), and the quantification of objective gene to β-actin levels was done by densitometry analysis.

Statistical analysis

All data were expressed as mean ± S.D. Results were analyzed by one-way analysis of variance

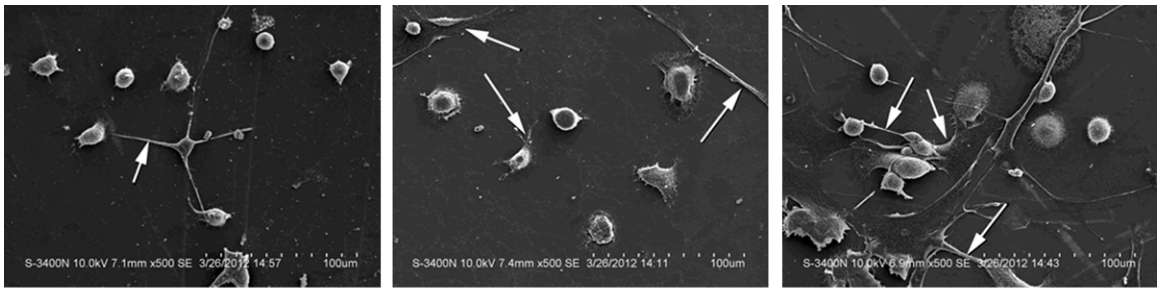

Figure 4. Effect of IFN-β on the ultrastructural of astrocytes. The astrocytes were treated with IFN-β (10^2 and 10^3 U/mL) for 72 h. The ultrastructural of astrocytes was observed by scanning electron microscopy (1:500). The arrow showed cell protrusions.

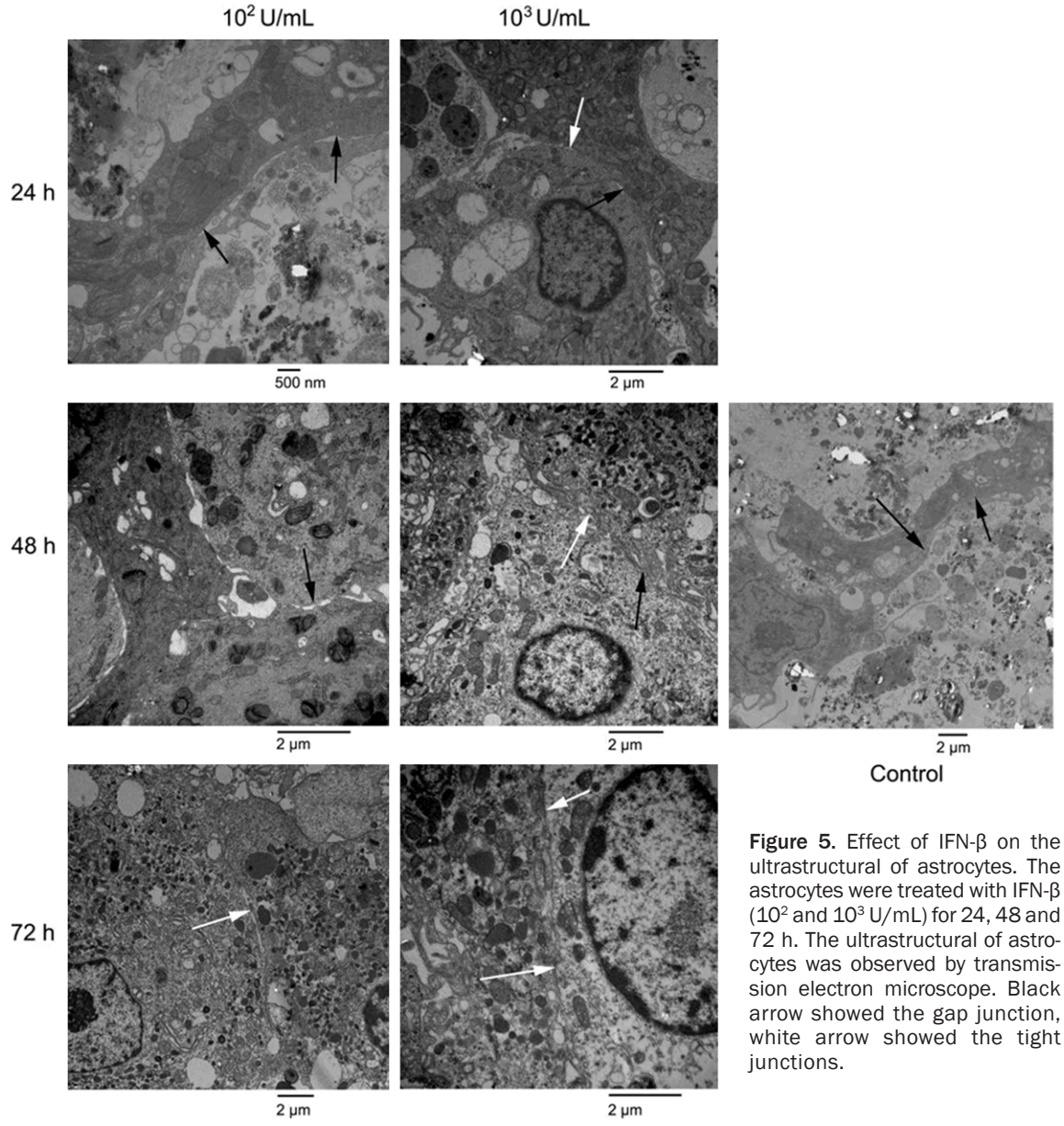

Figure 5. Effect of IFN-β on the ultrastructural of astrocytes. The astrocytes were treated with IFN-β (10^2 and 10^3 U/mL) for 24, 48 and 72 h. The ultrastructural of astrocytes was observed by transmission electron microscope. Black arrow showed the gap junction, white arrow showed the tight junctions.

Table 1. Analysis of OD value among different groups

Group	24 h	48 h	72 h
Control	0.168 ± 0.042	0.199 ± 0.014	0.21 3 ± 0.003
10^2 U/mL	0.16 3 ± 0.045	0.195 ± 0.021	0.219 ± 0.012
10^3 U/mL	0.184 ± 0.005	0.199 ± 0.011	0.219 ± 0.003

The astrocytes were treated with IFN-β (10^2 and 10^3 U/mL) for 24, 48 and 72 h. The cell viability was measured by MTT method. Data were expressed as mean ± S.D (n = 3).

(ANOVA) followed by Dunnett post hoc test. All data analyses were done using SPSS 17.0 statistical software. Significance level was set at $P < 0.05$.

Results

Effect of IFN-β on the morphology, cytoskeleton and cellular elasticity of AS

AS were identified with the method of comparison of the cytoplasm and nucleus staining. The result showed that about 90% of the cells were GFAP positive AS (data did not show). The cell morphology was observed by the immunofluorescence staining. As shown in **Figure 1**, there was no significant difference about the cell morphology and quantity among the different groups. Compared with the control group, the structure of intracellular microfilaments was more obvious under immunofluorescence microscope and the cell junctions were tighter after treatment with 10^2 and 10^3 U/mL IFN-β for 72 h.

The morphology of cells was observed by the atomic force microscope. As shown in **Figure 2**, compared with the control group, the cell junction was obviously increased, the cell morphology is more complete in treatment with 10^2 and 10^3 U/mL IFN-β for 72 h group. As shown in **Figure 3**, compared with the control group, the cells elastic modulus was significantly decreased in treatment with 10^2 and 10^3 U/mL IFN-β for 72 h group, which showed that the cell elasticity was increased by IFN-β. The morphology of cells was also observed by electron microscope. Compared with the control group, the cell junctions and cytoskeletons were obviously increased in treatment with 10^2 and 10^3 U/mL IFN-β for 72 h group compared with the control group (**Figures 4** and **5**).

Effect of IFN-β on the growth of AS

The cell optical density value (OD value) is proportional to the number of living cells, also is proportional to the cell growth rate. As shown in **Table 1**, there was no statistical significance about the OD values among the different groups ($P > 0.05$), which suggested treatment with IFN-β (10^2 and 10^3 U/mL) for 24, 48 and 72 h did not affect the growth of AS.

Effect of IFN-β on the expression of cytoskeleton and cellular elasticity associated protein in AS

The expressions of the cell structure and elasticity associated proteins in AS were tested by Western Blot, which included cofilin-1, GFAP, profilin-1 and vimentin. As shown in **Figure 6**, compared with the control group, the expressions of cofilin-1, GFAP, profilin-1 and vimentin in AS were significantly increased in treatment with 10^3 U/mL IFN-β for 48 h and 10^2 and 10^3 U/mL IFN-β for 72 h group (all $P < 0.05$).

Discussion

In recent years, AS have been obtained more and more attention of researchers as the most abundant cells in the CNS. With the deepening of the understanding, a variety of studies have been shown that except for the effector T cells in the autoimmune disease of CNS, AS also played an important role [9, 10]. AS form a three-dimensional regulatory network, carry out neurotransmitter metabolism and outstanding information conduction in the CNS to maintain the steady state of the CNS. Usually AS is in a quiescent state. When there is infection, ischemia, hypoxia, and brain trauma lesion, AS will be changed in the morphology and function, such as increase of GFAP expression, increase of cell processes, enhance of metabolism and increase of secreted cytokines and neurotrophic factors, which be called the gliosis. Damaged and degenerated neurons are surrounded with activated AS, which is conducive to the remodeling and repair of the damaged area in the CNS. The neurotrophic factors released by AS have an active role on microglia to repair myelin [11, 12].

This experiment was designed to apply different methods to observe the changes of cell morphology, proliferation, cell junctions and cell elasticity induced by IFN-β in AS. Our results showed that IFN-β might be therapeutic way on MS through the effect on AS. The possible reason may be the reconstruction of the cytoskel-

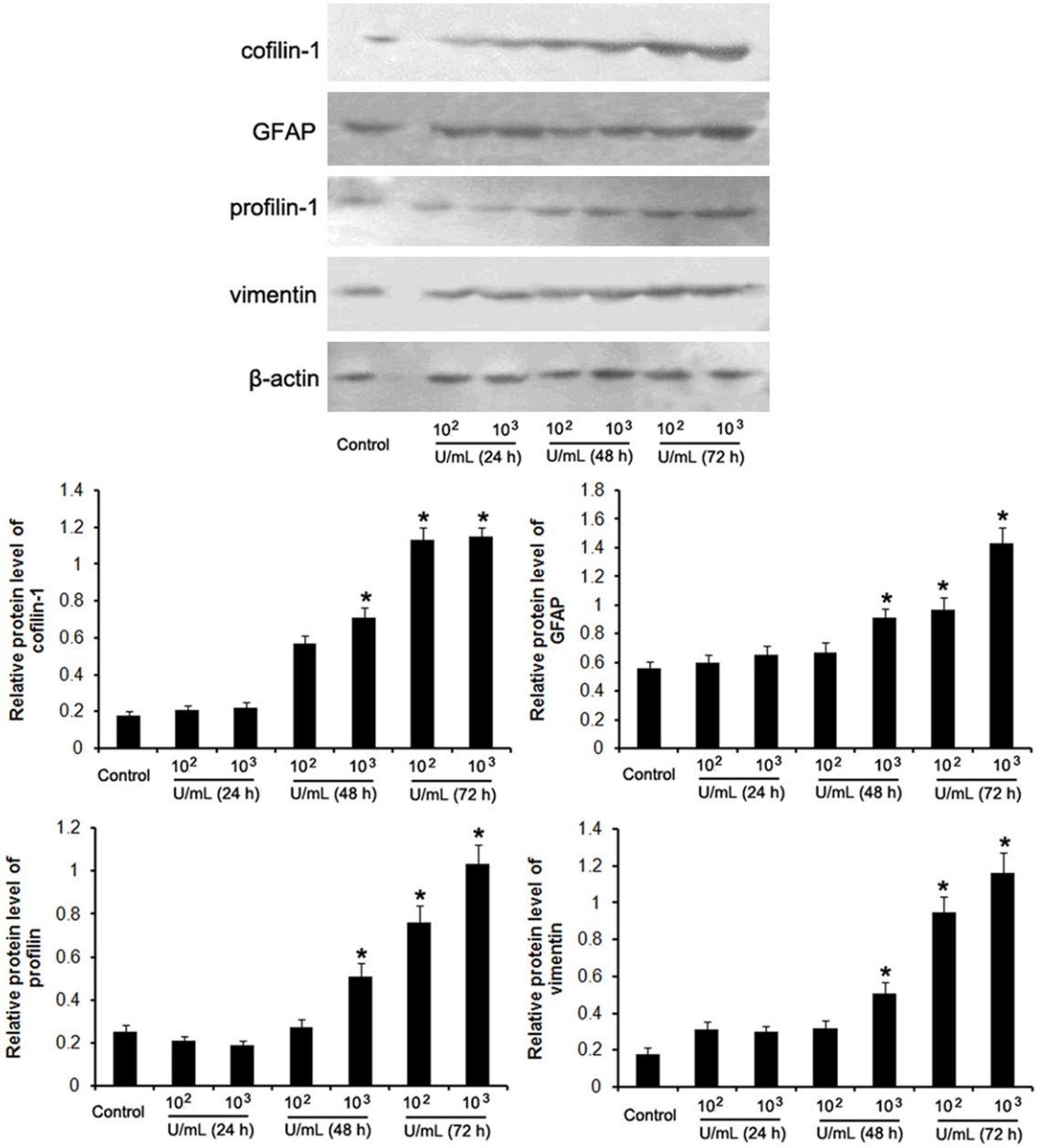

Figure 6. Effect of IFN-β on the expression of cytoskeleton and cellular elasticity associated protein in AS. The astrocytes were treated with IFN-β (10^2 and 10^3 U/mL) for 24, 48 and 72 h. The expression of protein was detected by Western Blot. Data were expressed as mean ± S.D (n = 3). *P < 0.05 compared with the control group.

eton, but not the effect on morphology and growth of AS. At the same time, the increased expression of GFAP may be positively related to the IFN-β dose and action time. At the late stage of IFN-β treatment, the degree of AS activation was increased which might lead to the gliosis and formation of the glial scar.

The cells morphology and surface structure of AS after the IFN-β treatment were observed with the fluorescence microscopy and atomic force microscope. The results showed that with increase of dose and time of IFN-β, GFAP expression was increased, indicating that the activation of AS was significantly enhanced with the increase of the drug concentration and time of action. However, cell morphology did not been significantly changed, but the intracellular fibers was increased, and cell integrity and cell elasticity was enhanced. The scanning

electron microscope and transmission electron microscope technology were furtherly to observe the cell junctions. The results showed that the cell junctions were obviously closer and the cells projections were obviously increased, which supplied the requirements for the transmission of information between cells, signal transduction and antigen delivery.

This experiment was designed to detect the effect of IFN-β on the survival and proliferation in AS. The results showed that IFN-β did not affect the cell growth state. However, some studies have shown that IFN-β not only promoted the proliferation of AS, but also inhibited the proliferation of AS by inhibiting the synthesis of nerve growth factor [13, 14]. Therefore, the further long-term culture is needed to explore the effect of IFN-β on the proliferation of AS for more explicit results. By the atomic force microscopy, the Young's modulus of cells was observed and calculated. The results showed that the Young's modulus was decreased with the increase of concentrations and duration of IFN-β, indicating that the cell elasticity was enhanced. These results suggested that IFN-β may play an important role in regulation of g elasticity and toughness in AS.

Proteomic analysis of whole-cell proteins showed that some proteins in the AS cytoskeleton play an important role, including cofilin-1, GFAP, profilin-1 and vimentin, and so on [15]. Cofilin is a kind of main actin depolymerization protein by phosphorylation/dephosphorylation to regulate the activity of highly conserved serine residues. This phosphorylation inhibits the activity of the protein and then affects the affinity of actin [16, 17]. Profilin-1, another actin-binding protein, catalyzes the conversion of monomers coupling with ADP or ATP, which activates the vitality of G-actin. Profiling-1 has an important role in signal transduction pathways and microfilament dynamics. It also combines with the monomer actin (G-actin), and then inhibits or promotes the assembly of microfilaments with a synergistic effect with cofilin-1 [18]. In our study, the effect of IFN-β on the cytoskeletal proteins in AS was observed, including cofilin-1 and profilin-1. The results showed that the expressions of ccofilin-1 and profilin-1 in AS were significantly increased by IFN-β, suggesting that IFN-β had an important role on the regulation of the flexibility and toughness in AS. It was reported that the muscle actin was damaged, the cytoskeleton was destructed, and cell elasticity decreased in AS of encephalomyelitis model [19, 20]. Therefore, our results elucidate that IFN-β may be the therapeutic approach for MS through the regulation of the elasticity and structure in AS.

The results showed that the expressions of GFAP and vimentin in AS were significantly increased by IFN-β. GFAP and vimentin are the landmark proteins and their expressions were increased in AS after the proliferation stimulation and brain injury [21]. After brain damage, AS activation has a two-way significance to the body. AS forms a glial barrier around the lesion. Although this barrier prevents axonal regeneration after injury, it also extends the next injury [22, 23]. Therefore, on the one hand, the GFAP expression in AS is increased after IFN-β treatment, which is important for the nerve repair and regeneration after CNS injury, on the other hand, the excessive activation of AS leads to the formation of the glial scar which blocks the axonal regeneration.

In summary, our study suggested that the expressions of microtubules and intermediate filaments in AS were increased, the projections of AS was increased, and the cell junction was more closely connected after treatment with IFN-β. Although the number of AS was unchanged after treatment with IFN-β, the increase of GFAP expression suggested that the activation of AS was increased, which meant the next injury time might be extended, on the other hand, excessive activation could lead to the generation of glial scar. So our study elucidate that IFN-β increases the flexibility and toughness of AS through up-regulation the expressions of cytoskeleton-elasticity associated protein. This study provides a new direction for the target in the treatment of MS through IFN-β treatment.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiaobo Yan, Department of Neurology, The Second Clinical Hospital of Harbin Medical University, Harbin 150086, China. Tel: 086-13633661618; 086-0451-86296489; E-mail: xiaoboyancn@163.com

References

[1] Sakguch S, Powrie F. Emerging challenges in regulatory T cell function and biology. Science 2007; 317: 627-9.

[2] Su S, Liu H. The association between multiple sclerosis and fracture risk. Int J Clin Exp Med 2014; 7: 4327-31.
[3] Cao L, Xue L, Luo DM. Lower serum bilirubin concentration in patients with migraine. Int J Clin Exp Med 2015; 8: 13398-402.
[4] van Strien ME, Drukarch B, Bol JG, van der Valk P, van Horssen J, Gerritsen WH, Breve JJ, van Dam AM. Appearance of tissue transglutaminase in astrocytes in multiple sclerosis lesions: a role in cell adhesion and migration? Brain Pathol 2011; 21: 44-54.
[5] Wang J, Jiao H, Stewart TL, Shankowsky HA, Scott PG, Tredget EE. Improvement in postburn hypertrophic scar after treatment with IFN-alpha2b is associated with decreased fibrocytes. J Interferon Cytokine Res 2007; 27: 921-30.
[6] Loma I, Heyman R. Multiple sclerosis: pathogenesis and treatment. Curr Neuropharmacol 2011; 9: 409-46.
[7] Rao YQ, Li J, Wang WJ. Effects of Gengnianchun on learning and memory ability, neurotransmitter, cytokines, and leptin in ovariectomized rats. Int J Clin Exp Med 2015; 8: 8648-60.
[8] Kohriyama T, Ochi K, Matsumoto M. Interferon-beta treatment for multiple sclerosis and predictors of response. Nihon Rinsho 2008; 66: 1119-26.
[9] Cai Q, Chen Z, Song P, Wu L, Wang L, Deng G, Liu B, Chen Q. Co-transplantation of hippocampal neural stem cells and astrocytes and microvascular endothelial cells improve the memory in ischemic stroke rat. Int J Clin Exp Med 2015; 8: 13109-17.
[10] Yang JF, Tao HQ, Liu YM, Zhan XX, Liu Y, Wang XY, Wang JH, Mu LL, Yang LL, Gao ZM, Kong QF, Wang GY, Han JH, Sun B, Li HL. Characterization of the interaction between astrocytes and encephalitogenic lymphocytes during the development of experimental autoimmune encephalitomyelitis (EAE) in mice. Clin Exp Immunol 2012; 170: 254-65.
[11] Xu X, Warrington AE, Bieber AJ, Rodriguez M. Enhancing CNS repair in neurological disease: challenges arising from neurodegeneration and rewiring of the network. CNS Drugs 2011; 25: 555-73.
[12] Xue Q, Liu Y, Qi H, Ma Q, Xu L, Chen W, Chen G, Xu X. A novel brain neurovascular unit model with neurons, astrocytes and microvascular endothelial cells of rat. Int J Biol Sci 2013; 9: 174-89.
[13] Detje CN, Lienenklaus S, Chhatbar C, Spanier J, Prajeeth CK, Soldner C, Tovey MG, Schlüter D, Weiss S, Stangel M, Kalinke U. Upon intranasal vesicular stomatitis virus infection, astrocytes in the olfactory bulb are important interferon Beta producers that protect from lethal encephalitis. J Virol 2015; 89: 2731-8.
[14] Latronico T, Branà MT, Gramegna P, Fasano A, Di Bari G, Liuzzi GM. Inhibition of Myelin-Cleaving Poteolytic Activities by Interferon-Beta in Rat Astrocyte Cultures. Comparative Analysis between Gelatinases and Calpain-II. PLoS One 2013; 8: e49656.
[15] Zheng J, Bizzozero OA. Accumulation of protein carbonyls within cerebellar astrocytes in murine experimental autoimmune encephalomyelitis. J Neurosci Res 2010; 88: 3376-85.
[16] Wuestefeld R, Chen J, Meller K, Brand-Saberi B, Theiss C. Impact of vegf on astrocytes: Analysis of gap junctional intercellular communication, proliferation, and motility. Glia 2012; 60: 936-47.
[17] de Graauw M, Cao L, Winkel L, van Miltenburg MH, le Dévédec SE, Klop M, Yan K, Pont C, Rogkoti VM, Tijsma A, Chaudhuri A, Lalai R, Price L, Verbeek F, van de Water B. Annexin A2 depletion delays EGFR endocytic trafficking via cofilin activation and enhances EGFR signaling and metastasis formation. Oncogene 2014; 33: 2610-9.
[18] Lee CK, Park HJ, So HH, Kim HJ, Lee KS, Choi WS, Lee HM, Won KJ, Yoon TJ, Park TK, Kim B. Proteomic profiling and identification of cofilin responding to oxidative stress in vascular smooth muscle. Proteomics 2006; 6: 6455-75.
[19] Fressinaud C, Berges R, Eyer J. Axon cytoskeleton proteins specifically modulate oligodendrocyte growth and differentiation in vitro. Neurochem Int 2012; 60: 78-90.
[20] Myhill S, Booth NE, McLaren-Howard J. Targeting mitochondrial dysfunction in the treatment of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)-a clinical audit. Int J Clin Exp Med 2013; 6: 1-15.
[21] Kipp M, Clarner T, Gingele S, Pott F, Amor S, van der Valk P, Beyer C. Brain lipid binding protein (FABP7) as modulator of astrocyte function. Physiol Res 2011; 60: 49-60.
[22] Miller WJ, Leventhal I, Scarsella D, Haydon PG, Janmey P, Meaney DF. Mechanically induced reactive gliosis causes ATP-mediated alterations in astrocyte stiffness. J Neurotrauma 2009; 26: 789-97.
[23] Kamphuis W, Kooijman L, Orre M, Stassen O, Pekny M, Hol EM. GFAP and vimentin deficiency alters gene expression in astrocytes and microglia in wild-type mice and changes the transcriptional response of reactive glia in mouse model for Alzheimer's disease. Glia 2015; 63: 1036-56.