Biomedicine & Pharmacotherapy 98 (2018) 63–68

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Lin28a functionally modulates bupivacaine-induced dorsal root ganglion neuron apoptosis through TrkA activation

Liangsheng Yu[a], Yuanxu Jiang[b], Bin Tang[c,*]

[a] Department of Anesthesiology, Huangshi AiKang Hospital, Huangshi, 435000, China
[b] Department of Anesthesiology, People's Hospital of Shenzhen, Shenzhen, 518000, China
[c] Department of Anesthesiology, Huangshi Maternity and Children's Health Hospital, Edong Health care Group, 80 South Guilin Rd., Huangshi, 435000, China

ARTICLE INFO

Keywords:
Lin28a
DRGN
Bupivacaine
Apoptosis
Neuron

ABSTRACT

Purpose: Lin-28 Homolog A gene (Lin28a) is an important regulator in nerve system. In this study, we investigated the functional mechanism of Lin28a during the process of bupivacaine (BUP)-induced neuronal apoptosis of spinal cord dorsal root ganglion neurons (DRGNs).

Methods: Young mouse DRGNs were cultured *in vitro* and treated with series concentrations of BUP. Apoptosis was evaluated by TUNEL assay. Corresponding Lin28a mRNA and protein expressions were evaluated by qRT-PCR and western blot (WB) assays. Lin28a was downregulated by siRNA and its effect on BUP (5 mM)-induced DRGN apoptosis was measured by qRT-PCR, WB and TUNEL assays, respectively. Alternatively, Lin28a was upregulated in DRGNs. It's effect on BUP (0.1 mM)-induced DRGN apoptosis was also measured. Finally, WB was used to examine Caspase-3/9 and TrkA protein expressions in Lin28a-downregualted and BUP-injured DRGN to explore Lin28a-associated signaling pathways.

Results: In DRGN *in vitro* culture, 0.1 mM BUP induced moderate neuronal apoptosis while 5 mM BUP induced significant apoptosis. Lin28a mRNA and protein were both upregulated by BUP, in concentration-dependent manner. Functional assays showed that Lin28a downregulation rescued 5 mM BUP-induced neuronal apoptosis, whereas Lin28a upregulation aggravated 0.1 mM BUP-induced neuronal apoptosis in DRGNs. WB showed that Lin28a downregulation reduced Caspase-3/9 proteins and activated TrkA through phosphorylation in BUP-injured DRGNs.

Conclusion: Lin28a is a potent regulator in BUP-induced neuronal apoptosis in DRGNs. The apoptotic protection by Lin28a inhibition is likely through the activation of TrkA signaling pathway.

1. Introduction

Evidences from both clinics and laboratories showed that, local induction of anesthetic reagents, such as mepivacaine, ropivacaine, lidocaine or bupivacaine (BUP) might lead to neuronal apoptosis, neurological complications, or even permanent nerve injury [1–5]. It was also demonstrated that BUP was more toxic than procaine or mepivacaine to induce growth cone collapses, neurite degeneration and subsequent neuronal apoptosis in cultured central neurons [3]. Moreover, while the complete molecular signaling pathways of local anesthetics-induced neurotoxicity were yet to be defined, several molecular mechanisms, such as mitochondrial or cytosolic calcium overflow [5,6], or epigenetic regulation by microRNAs [7,8] may be responsible for the cause of neuronal apoptosis or the recovery/protection against neurotoxicity in a variety of central and peripheral neurons.

Among many of the neuronal populations subject to local anesthetics (LA)-induced neurotoxicity, studies had shown that spinal cord dorsal root ganglion neurons (DRGNs) were vulnerable to those neuronal injuries with significant LA-induced apoptosis [2,9]. On the other hand, cultured DRGNs served as an excellent and relative-simple *in vitro* model to study LA-induced neuronal complications, as various signaling pathways, including neurotrophins, glycogen synthase kinase-3 (GSK-3) signaling pathways or microRNAs had been shown to exert protective or regenerative effects after DRGNs suffered LA-induced neurotoxicity [7,9,10].

Gene Lin-28 Homolog A (Lin28a), originally identified in genetic screening in *C. elegans* [11], has been demonstrated to be a critical factor regulating self-renewal capabilities in several stem cell lineages [12–14]. In addition, Lin28a was shown to play important role in regulating oncogenic development in various human cancers [15–17]. In neuronal populations, evidence started to emerge that Lin28a may also be an active factor in regulating neuronal differentiation in both

* Corresponding author.
E-mail address: tangb118@aol.com (B. Tang).

https://doi.org/10.1016/j.biopha.2017.11.142
Received 23 August 2017; Received in revised form 26 November 2017; Accepted 27 November 2017
0753-3322/ © 2017 Published by Elsevier Masson SAS.

cell lines and *in vitro* models of neurodegenerative diseases [18,19]. Specifically, Lin28a was shown to be involved in the process of spinal cord injury [20]. Lin28a was dynamically regulated by contusion injury and closely associated with NF-kappaB signaling pathway and lipopolysaccharide-induced astrocytes inflammatory responses [20]. However, it is still unknown whether Lin28a was involved in the process of LA-induced neuronal injury in spinal cord DRGNs.

2. Materials and methods

2.1. Ethic statement

In this study, all protocols were reviewed and approved by the institutional animal care and use committees (IACUCs) and Clinical Study & Ethics Committees at Huangshi AiKang Hospital & Huangshi Maternity and Children's Health Hospital in Hangshi, and People's Hospital of Shenzhen in Shenzhen, China. All procedures were carried out according to the 8th edition of the Guide for the Care and Use of Laboratory Animals [21].

2.2. Animal and explant of dorsal root ganglion neurons

Adult male C57BL/6 mice (8-week old) were purchased from Branch of National Breeder Center of Rodents (Beijing, China). The explant of dorsal root ganglion neurons (DRGNs) was prepared according to the methods described previously [7,8]. Briefly, mice were anesthetized and sacrificed. L4-L5 portion of the lumbar spinal cord was extracted and quickly transferred to a glass petri-dish (Corning, USA) containing hanks balanced salt solution (HBSS, Sigma, Shanghai, China) at 4 °C. The region of dorsal root ganglia was extracted, and then dissociated in 0.1% Trypsin solution (Sigma, Shanghai, China) for 20 min at 37 °C. Then, Dulbecco Modified Eagle Medium (DMEM, Gibco, USA) containing 10% fetal bovine serum (FBS, Sigma, Shanghai, China) was added into Trypsin solution and cell clumps were centrifuged for 5 min at 800 rpm at room temperature. After removing supernatant, cell pellets were suspended in a 6-well plate containing serum-free DMEM medium and 1X B27 supplement (ThermoFisher, USA) at 37 °C to enrich neuronal population. 48 h later, attached cells (most of them were dorsal root ganglion neurons (DRGNs)) were collected and re-plated in a new 6-well plate containing DMEM, 10% FBS, 1X B27 supplement, 0.2 mM l-Glutamine (ThermoFisher, USA) and 100 U/ml penicillin/streptomycin (ThermoFisher, USA), and were maintained in an environment with 5% CO_2 at 37 °C.

2.3. In vitro application of bupivacaine

The model of applying bupivacaine (BUP) among DRGN explants was established according to the method described previously [8]. Briefly, DRGN explant was incubated with different concentrations of BUP between 0.001 and 10 mM for 2 h. After that, explant was changed to fresh medium without BUP for 24 h.

2.4. Apoptosis assay

BUP-induced neuronal apoptosis among DRGN explants was measured using a Click-iT™ Plus TUNEL Assay for In Situ Apoptosis Detection, Alexa Fluor™ 594 dye (ThermoFisher, USA) according to the manufacturer's instruction. In addition, a rabbit polyclonal NeuN antibody (1:500, Abcam, USA) was used alongside apoptosis assay to identify the nuclei of DRGNs. After that, the explants were examined by a DMi1 inverted fluorescent microscope (Leica, Germany). Healthy DRGNs were identified as those NeuN-positive but TUNEL-negative cells. The relative percentages of healthy DRGNs were then normalized to the ones under control conditions.

2.5. RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was collected from DRGN explant using a PicoPure™ RNA Isolation Kit (Applied Biosystems, USA), followed by a TRIzol™ Plus RNA Purification Kit (Invitrogen, USA) according to the manufacturer's instructions. Then, a High-Capacity RNA-to-cDNA™ Kit (Applied Biosystems, USA) was applied to obtain complementary DNAs (cDNAs). Quantitative real-time PCR (qRT-PCR) was carried out using an ABI PRISM 7900 Sequence Detection System (Applied Biosystems, USA). A PowerUp SYB Green Master Kit (Applied Biosystems, USA) was then used according to the manufacturer's instruction with mouse-specific lin28a primer pair. The internal control used GAPDH gene. Relative lin28a mRNA levels were then quantified using the $2^{-\Delta CtCt}$ method.

2.6. Western blot assay

Total protein was extracted from DRGN explant using a CyQUANT™ cell Lysis Buffer (Invitrogen, USA) according to the manufacturer's instruction. After purification, 30 ng proteins were separated on a 10% gradient SDS NuPAGE gel (Invitrogen, USA) and then blotted onto PVDF membranes (Invitrogen, USA). After 1 h blocking, the membranes were treated with rabbit polyclonal Lin28a antibody (1:500, Abcam, USA), rabbit polyclonal beta-actin antibody (1:10,000, Abcam, USA), rabbit polyclonal caspase-3 (Casp-3, 1:2,000, Abcam, USA), rabbit polyclonal caspase-9 (Casp-9, 1:2,000, Abcam, USA), rabbit polyclonal Tropomyosin receptor kinase A (TrkA, 1:1,000, Abcam, USA) and rabbit polyclonal phosphor-TrkA (p-TrkA, 1:200, Abcam, USA) antibodies for 24 h at 4 °C. Then, membranes were treated with anti-rabbit HRP-conjugated secondary antibodies (110,000, Abcam, USA) for 30 min at 37 °C. The blots were imaged and quantified using an enhanced chemiluminescence system (Amersham Pharmacia Biotech, USA) according to the manufacturer's instruction.

2.7. Lin28a downregulation assay

Mouse specific Lina28a small interfering RNAs (Si-Lin28a), and a corresponding non-specific mouse siRNA (Si-NS) were purchased from RiboBio (RiboBio Biotechnology, Guangzhou, China). DRGN explant was transfected with 2 μM Si-NS or Si-Lin28a along with Lipofectamine 2000 reagent (Sigma, Shanghai, China) for 24 h. QRT-PCR was then carried out to examine Lin28a downregulation efficiency.

2.8. Lin28a upregulation assay

Whole mouse Lin28a gene was amplified from a mouse-spinal-cord cDNA library and cloned into a mammalian overexpression vector pcDNA/3.1 (Promega, USA) to generate the Lin28a upregulating vector (pc-Lin28a). An empty pcDNA/3.1 vector was used in this study as non-specific upregulating vector (pc-NS). DRGN explant was transfected with 250 ng pc-NS or pc-Lin28a along with Lipofectamine 2000 reagent (Sigma, Shanghai, China) for 48 h. QRT-PCR was then carried out to examine Lin28a upregulation efficiency.

2.9. Statistical analysis

All experiments were carried out by at least triplicates. Averaged data was presented as mean ± S.E.M.. All statistical analysis was carried out using an unpaired student's *t*-test on a Prizm Software (GraphPad Software, USA). Significant difference was declared while a $P < 0.05$.

Download English Version:

https://daneshyari.com/en/article/8525860

Download Persian Version:

https://daneshyari.com/article/8525860

Daneshyari.com