Journal of Biomechanics xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

The shear wave elastic modulus and the increased nuclear factor kappa B (NF-kB/p65) and cyclooxygenase-2 (COX-2) expression in the area of myofascial trigger points activated in a rat model by blunt trauma to the vastus medialis

Hengyong Lv [a], Zhen Li [a], Tiantian Hu [b,c], Yuexiang Wang [d], Jinpeng Wu [a,*], Yingxin Li [a,*]

[a] Laser Medicine Laboratory, Institute of Biomedical Engineering, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300192, PR China
[b] Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education and Guangdong Province, College of Optoelectronic Engineering, Shenzhen University, Shenzhen 518060, PR China
[c] School of Medicine, Shenzhen University, Shenzhen 518060, PR China
[d] Department of Ultrasound, Chinese People's Liberation Army General Hospital, Beijing 100853, PR China

ARTICLE INFO

Article history:
Accepted 27 October 2017
Available online xxxx

Keywords:
Myofascial trigger points
Inflammatory cytokines
Shear wave elastography
Elastic modulus

ABSTRACT

We aimed to elucidate the increased inflammatory cytokines expression such as nuclear factor kappa B (NF-kB/p65), cyclooxygenase-2 (COX-2), and voltage-gated calcium channels (VGCC) in the area of activated myofascial trigger points (MTrPs) in a rat model by blunt trauma to the vastus medialis and to evaluate the feasibility of a quantitative analysis of muscle elastic modulus using shear wave elastography (SWE). Twelve 7-week-old male SD rats were divided into normal (NM, n = 6) and model groups (MO, n = 6). In the MO group, MTrPs were activated with a blunt strike to the left vastus medialis and subsequent eccentric exercise for 8 weeks. After 4 weeks of rest, the elastic modulus in the focal site was evaluated using SWE. Electromyography (EMG) data were collected at MTrPs and muscle tissues were evaluated for expression of nuclear factor kappa B (NF-kB/p65), cyclooxygenase-2 (COX-2) protein, and voltage-gated calcium channels (VGCC). The number of the palpable taut bands; EMG frequency and amplitude; elastic modulus values; and NFkB/p65, COX-2, and VGCC expression levels were significantly higher for the left focal area in the MO group compared to those for the NM group (p's < 0.05). These findings suggest that elastic modulus measurement using ultrasound SWE may be effective in evaluating MPS. In addition, increased COX-2, NFkB/p65, and VGCC expression may expand the integrated hypothesis of trigger points.

1. Introduction

Myofascial pain syndrome (MPS) is a muscular dysfunction involving pain originating from myofascial trigger points (MTrPs) located in taut bands of skeletal muscle (Simons et al., 1999; Wheeler and Aaron, 2001). MTrPs can be found in a tender, taut band and consist of a group of contracted muscle fibers that can be palpated in the muscle (Fricton et al., 1985). The main clinical criteria, that pain or referred pain is felt when the taut band is stimulated either mechanically or by compression, is controversial, as the diagnosis for the taut band depends on the clinician's interviewing and examination skills. Thus, locating the taut band is important to confirm the diagnosis of myofascial pain syndrome. Shear-wave elastography (SWE) is a novel noninvasive dynamic and real-time ultrasound (US) imaging technology. The main parameter of SWE, the elastic modulus (E, in kilopascals), can objectively quantify tissue mechanical properties (Eby et al., 2013). Maher et al., 2013 showed that the elastic modulus of the upper trapezius muscle with MTrPs was reduced after treatment. Thus, an objective quantification of the muscle stiffness for MTrPs is possible.

Many factors can activate MTrPs including spine pathology, trauma, repetitive strain, exposure to cumulative physical deconditioning, and postural dysfunction (Shah et al., 2008). These factors induce a chemical inflammatory response in the muscle tissue. Shah et al., 2008 reported that trigger zone biochemical changes were the reason for persistent muscle fiber contraction in the taut band. Moreover, the transcription factor, nuclear factor-kappa B/P65 (NF-kB/P65), is known to play a pivotal role in the inflamma-

* Corresponding authors.
E-mail addresses: microkn@126.com (J. Wu), yingxinli2005@126.com (Y. Li).

https://doi.org/10.1016/j.jbiomech.2017.10.028
0021-9290/

tory processes (Hayden and Ghosh, 2008). NF-kB regulates the expression of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase, pro-inflammatory cytokines, and dynorphin, which initiate the inflammatory response (O'neill and Kaltschmidt 1997; Barnes and Adcock, 1997). In skeletal muscle, voltage-gated calcium channels (VGCCs) function as a voltage sensor excitation-contraction (EC) coupling located in the sarcoplasmic reticulum (SR), which release calcium (Ca^{2+} ions) out of the SR to regulate skeletal muscle contraction (Simons et al., 1999; Gehlert et al., 2015; Tuluc et al., 2009). An increase in NF-kB/P65, COX-2, and VGCC levels can indicate the production of inflammatory mediators, modulate pain transmission, and the muscle stiffness of MTrPs (Bonizzi and Karin, 2004).

Therefore, an experimentally controlled study involving animals may be particularly informative in the investigation of biochemical alterations related to pain (including NF-kB/P65, COX-2, and VGCC) and muscle stiffness as assessed by SWE. The aims of the present study were to elucidate the increased inflammatory cytokines expression such as nuclear factor kappa B (NF-kB/p65), cyclooxygenase-2 (COX-2), and voltage-gated calcium channels (VGCC) involved in MTrP activation using SWE in a rat model, and to evaluate the feasibility of a quantitative analysis of the muscle elastic modulus.

2. Methods

The study utilized an experimental prospective design with two parallel groups: one control group and one experimental group. Observers were blinded during the SWE analyses. All experiments were approved by the local animal welfare committee.

2.1. Animal modeling and care

Seven-week-old male Sprague Dawley (SD) rats (body weight, 220–250 g) were divided into two groups: (1) the normal group (NM, n = 6), and (2) the model group (MO, n = 6).

In the MO group, the MTrPs were activated with a blunt strike to the left vastus medialis (VM) combined with eccentric exercise for 8 weeks as in Huang et al., 2013. The rats on the MO group were anaesthetised with an injection of 3 ml/kg 10% chloral hydrate into the abdominal cavity, and placed on the board of a self-made striking device (Fig. 1). Specifically, the site of the proximal vastus medialis (VM) of the left hind limb was marked and the blunt strike was delivered to a marked position by a stick dropped from a height of 20 cm on the left VM . The stick was 50 cm long, which cone head was sized less than 1 cm^2. The kinetic energy of the blunt strike was 2.352 J.

The eccentric exercise involved rats running on a treadmill for 90 min at a 16° downward angle at a speed of 16 m/min. The modeling intervention lasted for 8 weeks, during which, the blunt strike was performed on the first day of every week, followed by eccentric exercise on the next day. Following the modeling intervention and 4 weeks of rest, SWE was performed on all rats. Finally, a pathophysiologic study was performed to investigate the inflammation mechanism of MTrPs, including NF-kB, COX-2, and VGCC assessments. An analysis of the electromyography (EMG) and number of taut bands was used to evaluate activated MTrPs. All the rats were raised in a conventional laboratory (temperature: 20–25 °C; humidity: 40–70%). Water and food were provided freely.

2.2. Ultrasound imaging with SWE

Ultrasonographic elastography was performed by an experienced radiologist using an Aixplorer ultrasound system (SuperSonic Imagine, Aix, France) with a SuperLinear 15–4 transducer at 4–15 MHz. The testing (examination) was performed on the muscles of the left hind limbs, including the VM, gracilis (GR), adductor longus (AL), adductor magus (AM), and adductor femoris (AF) muscles. All rats were anaesthetized with an injection of 10% chloral hydrate into the abdominal cavity at a concentration of 4 mL/kg while lying in a horizontal position with limbs relaxed. Based on observations in the conventional ultrasound scanning mode (B mode), the mode was switched to the supersonic shear imaging mode (SSI mode) to find the area of interest. When a stable color-coded map was obtained, the elastic modulus (Young's modulus) was recorded. The dynamic range of the color map was set at 180 kPa and the region-of-interest (ROI) consisted of a circular area with a diameter of 2 mm. The maximum, minimum, and average/mean of the elastic modulus were obtained for the ROIs.

The ROIs were chosen by an experienced radiologist who checked the muscle and used the elastography mode to position/locate the left focal area (MLF) of the MTrPs. When the MLF was determined, the left adjacent area (MLA) within the same muscle was also chosen as an ROI (Fig. 2). In addition, the same positions in the right limbs were selected as controls, including the right control area (MRF) and the right adjacent control area (MRA) within the same muscle. The same areas of the NM group were chosen as the ROIs, which were named as the NF (left control area) and NA (the right adjacent control area). Values were averaged across 3 measurements performed in each condition for statistical analysis.

2.3. EMG and biomechanical testing

EMG data were recorded using an EMG device (YRKJ-X200X, Zhuhai, China). After all rats were anaesthetized, the muscles of the left hind limbs were completely exposed by surgery. An experienced clinician palpated the muscles to count the taut bands and mark the positions. Electrodes were inserted into the taut bands to determine whether there was an active MTrP (Huang et al., 2013). If an active MTrP was found, the EMG data was recorded. After the EMG data was obtained, a muscle biopsy of the active MTrPs was performed for biomechanical testing. As a control, EMG data were recorded in the same positions in the NM rats as in the MO rats.

2.4. Enzyme-linked immunosorbent assays for VGCC, NFkB/p65, and COX2

Serum samples were collected from the muscle tissue homogenate of five randomly selected animals of the MO and NM groups, and stored at −80 °C until assayed. The levels of serum VGCC, NFkB/p65, and COX2 were assessed using enzyme-linked immunosorbent assay (ELISA) kits (VGCC: rat voltage-gated calcium channel ELISA kit, cat. No. E-31586, Cheng Lin Biological Technology Co., Ltd, Beijing, China; NFkB/p65: nuclear factor kappa B ELISA kit, cat. No. SEB824Ra, USCN Business Science Co., Ltd, Wuhan, China; COX2: prostaglandin endoperoxide synthase 2 ELISA kit, cat. No. SEA699Ra, USCN Business Science Co., Ltd, Wuhan, China). The absorbance of a standard solution and experimental samples were read with a 450-nm filter using an automatic enzyme standard instrument (Thermo Fisher Scientific, multiscan MK3, USA). Protocols were performed according to the literature and the manufacturer's instructions.

2.5. Western blots for NFkB/p65 and COX2

Protein was extracted from muscle tissue homogenates (using the same animals as in the ELISA testing) for western blot detection with a sodium dodecyl sulfate (SDS) lysis buffer. Protein amounts were estimated in the classical Bradford manner. The specific

Please cite this article in press as: Lv, H., et al. The shear wave elastic modulus and the increased nuclear factor kappa B (NF-kB/p65) and cyclooxygenase-2 (COX-2) expression in the area of myofascial trigger points activated in a rat model by blunt trauma to the vastus medialis. J. Biomech. (2017), https://doi.org/10.1016/j.jbiomech.2017.10.028