Experimental Neurology 291 (2017) 44–50

Contents lists available at ScienceDirect

Experimental Neurology

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

Research Paper

Effects of liposome-based local suppression of nerve growth factor in the bladder on autonomic dysreflexia during urinary bladder distention in rats with spinal cord injury

Katsumi Kadekawa [a,c,d], Tsuyoshi Yoshizawa [a], Naoki Wada [a], Takahiro Shimizu [a], Tsuyoshi Majima [a], Pradeep Tyagi [a], William C. de Groat [b], Kimio Sugaya [c], Naoki Yoshimura [a,b,*]

[a] Department of Urology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[b] Department of Pharmacology & Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[c] Southern Knights' Laboratory, Okinawa, Japan
[d] Okinawa Kyodo Hospital, Okinawa, Japan

ARTICLE INFO

Article history:
Received 11 August 2016
Received in revised form 11 January 2017
Accepted 31 January 2017
Available online 4 February 2017

Keywords:
Autonomic dysreflexia
Spinal cord injury
Liposome NGF antisense
Rat

ABSTRACT

Purpose: To examine (1) whether spinal cord injury (SCI) time-dependently increases the severity of autonomic dysreflexia (AD) and expression levels of bladder nerve growth factor (NGF) protein, and (2) whether local suppression of NGF in the bladder improves SCI-induced AD in rats.
Materials and methods: SCI was produced by the transection of the T2/3 spinal cord in female Sprague-Dawley rats. At 4 or 8 weeks after SCI, differences in the mean arterial blood pressure (ΔMAP) and heart rate (ΔMHR) during graded increases in intravesical pressure to 20, 40 and 60 cm H_2O from those before bladder distention and NGF protein levels in the bladder wall were evaluated in spinal intact and SCI rats under urethane anesthesia. Seven weeks after SCI liposome-NGF antisense conjugates were administered intravesically to the animals. At 1 week after intravesical treatment (8 weeks after SCI), ΔMAP and ΔMHR during bladder distention and bladder NGF protein expression were evaluated.
Results: The ΔMAP and ΔMHR were increased in a graded manner in response to bladder distention at intravesical pressures of 20, 40 and 60 cm H_2O in SCI rats. These AD-like cardiovascular responses and NGF protein expression in the bladder mucosal and muscle layers were increased after SCI in a time-dependent manner. The liposome-NGF antisense treatment significantly reduced the NGF protein overexpression in the mucosal layer of SCI rat bladder and reduced ΔMAP and ΔMHR elicited by bladder distention.
Conclusions: These results indicate that the duration of the post-SCI recovery period affects the severity of AD induced by bladder distention as well as the level of bladder NGF protein, and that local suppression of NGF expression in the bladder reduces SCI-induced AD. Thus, Intravesical application of liposome-NGF antisense conjugates can be a new effective therapy for bladder distention-induced AD after SCI.

1. Introduction

Autonomic dysreflexia (AD) is a serious complication in persons with spinal cord injury (SCI) above the mid-thoracic spinal cord level. It is a potentially life-threatening medical emergency that induces uncontrolled sympathetic activity resulting in hypertension and bradycardia, and the source of stimulation initiating AD is bladder distention in up to 85% of cases, followed by colorectal distention in 13% of cases (Furusawa et al., 2011; Khastgir et al., 2007; Krassioukov et al., 2009). Clinically, episodes of AD usually occur within the first 6 to 12 months after injury (Colachis, 1991), and the blood pressure response during bladder distention induced during urodynamics or cystoscopy, is more severe after post SCI recovery periods >2 years than after recovery periods <2 years (Liu et al., 2013; Otani et al., 1985). Neurogenic lower urinary tract dysfunction, after SCI, presents with detrusor overactivity and detrusor-sphincter dyssynergia (DSD), which result in inefficient voiding and bladder wall tissue remodeling such as hypertrophy and fibrosis (de Groat and Yoshimura, 2010; Kadekawa et al., 2016). It has been demonstrated that, in SCI rats with spinal cord transection at the T4 level, DSD and AD are correlated, as evidenced by increases of electromyography activity of pelvic floor muscles during voiding contraction, elevations of mean blood pressure and decreases of mean heart rate at cystometric capacity (Rivas et al., 1995). Therefore, inefficient voiding and high intravesical pressure, induced by DSD after SCI, are likely to be involved in the emergence of AD. In addition, the temporal changes in AD during colorectal distention in SCI rats with T3

* Corresponding author at: Department of Urology, University of Pittsburgh School of Medicine, Suite 700, Kaufmann Medical Bldg., 3471 Fifth Ave., Pittsburgh, PA 15213, USA.
E-mail address: nyos@pitt.edu (N. Yoshimura).

http://dx.doi.org/10.1016/j.expneurol.2017.01.014
0014-4886/

transection has been shown by time-dependent increases in arterial pressure by 22 ± 3 and 49 ± 5 mm Hg accompanied by bradycardia at 7 and 30 days after SCI, respectively (Krassioukov and Weaver, 1995). Thus, it is assumed that the severity of AD may be time-dependent after SCI; however, a clinically relevant animal model of bladder distention-induced AD, which shows the time-dependent progression after SCI, has not been established.

A major mechanism underlying the emergence of AD is the development of a vesico-cardiovascular reflex triggered by activation of bladder afferents during bladder filling, which facilitates sympathetic nerve outflow via spinal reflexes below the level of injury and causes widespread vasoconstriction and hypertension that then triggers bradycardia due to activation of the vagal nerve-mediated baroreceptor reflex (Blackmer, 2003). Our recent study in SCI rats also revealed that AD induced by bladder distention is mediated by activation of resiniferatoxin-sensitive C-fiber afferents and is accompanied by increased expression of nerve growth factor (NGF) in the bladder wall and increased transient receptor potential (TRP) V1 channels in bladder afferent neurons (Yoshizawa et al., 2015). NGF overexpression in the bladder wall as well as in the spinal cord and dorsal root ganglia (DRG) after SCI is thought to be a major contributor to the induction of bladder afferent hyperexcitability and bladder overactivity (Seki et al., 2002; Yoshimura et al., 2006) as well as to AD during colon distention (Krenz et al., 1999). In addition, intravesical botulinum toxin treatment reportedly reduces arterial pressure responses during bladder contractions in association with a decreased NGF expression in the bladder and DRG of SCI rats (Elkelini et al., 2012). Taken together, these findings suggest that suppression of NGF expression in the bladder wall after SCI could reduce bladder afferent hyperexcitability, leading to a reduction in AD induced by a vesico-cardiovascular reflex during bladder distention.

A recent randomized double-blind placebo controlled phase 2 study, which examined the effects of tanezumab, an NGF monoclonal antibody, on clinical symptoms in patients with bladder pain syndrome showed that systemic application of the antibody significantly improved the symptoms (Evans et al., 2011); however, another clinical trial reported systemic adverse effects such as paresthesia, hypoesthesia and arthralgia, which led the FDA to terminate other trials using systemic application of NGF antibodies (Wood, 2010). Therefore, the site-specific reduction of NGF would be desirable to reduce the intrinsic toxicity from systemic blockade of NGF.

We recently reported that intravesical treatment with liposome-NGF antisense conjugates can deliver the NGF antisense to the bladder urothelium (Kashyap et al., 2013) and suppress the level of urothelial NGF expression as well as bladder overactivity in rats with acetic acid-induced cystitis (Kashyap et al., 2013) or experimental colitis (Kawamorita et al., 2016). Therefore, we hypothesized that local suppression of NGF expression in the bladder wall after SCI using intravesical liposome-based delivery techniques could also reduce bladder afferent hyperexcitability, leading to the improvement of AD after SCI. Thus, the present study was performed to examine; (1) whether SCI time-dependently increases the severity of vesico-cardiovascular hypertensive reflexes during bladder distention and expression levels of bladder NGF protein and (2) whether local suppression of NGF in the bladder improves bladder distention-induced AD in SCI rats.

2. Materials and methods

2.1. Animal preparation

Adult female Sprague–Dawley rats weighing 280–368 g (5 to 10 months old) with spinal intact (n = 6) and spinal cord injury (SCI) (n = 40) were used according to the experimental protocol approved by the University of Pittsburgh Institutional Animal Care and Use Committee. In SCI groups, the T2/3 spinal cord was transected under isoflurane anesthesia. Eight SCI rats per each of five experimental groups were prepared because of possible attrition due to premature death of some animals after high thoracic-level SCI. Using sharp microscissors, muscle and fascia between T2 and 3 vertebras were dissected, and the dura and spinal cord were transected completely with an aid of a surgical stereomicroscope. To ensure complete transection of the spinal cord, the tip of a 23G needle was inserted into the inner space between the exposed vertebrae. The overlying muscles and skin were then sutured. Buprenorphine (Tocris) (0.05 mg/kg, bid) was injected subcutaneously for 3 days postoperatively as an analgesic. Postoperatively the bladder was emptied by abdominal compression twice a day until reflex voiding recovered and once a day afterwards until the final experiment. The animals were treated with ampicillin (100 mg/kg, subcutaneously) for 5 days after surgery, and this treatment was continued twice a week afterwards to prevent urinary tract infection.

2.2. Evaluation of vesico-cardiovascular reflexes during bladder distention after SCI

The rats were divided into 3 groups; (1) spinal intact (n = 6), (2) 4 weeks after SCI (SCI 4 weeks, n = 6) and (3) 8 weeks after SCI (SCI 8 weeks, n = 7) groups. Two rats died prematurely in the 4 weeks group and one in the 8 weeks groups. These rats were anesthetized with urethane (0.9 g/kg subcutaneously) aided with isoflurane anesthesia. The dose of urethane was determined by our previous study (Yoshizawa et al., 2015) and preliminary experiments to suppress the body movement in spinal intact rats and spastic movements of the hind limbs in SCI rats during bladder distention. A 3Fr catheter (Hakko Co., Ltd., Nagano, Japan) filled with saline containing heparin was inserted into the common carotid artery to record the mean arterial pressure (MAP) and the mean heart rate (MHR). The bladder was exposed via a lower abdominal incision, and bilateral ureters were cut and the distal ends were ligated to prevent vesicoureteral reflux during bladder distention (Sugata, 1980). A polyethylene catheter (PE-160, Clay-Adams, Parsippany, NJ) was inserted through the bladder dome and a purse suture was placed tightly around the catheter. The urethral orifice was closed with glue to prevent fluid leakage (Fig. 1-A). Thereafter, isoflurane anesthesia was turned off and the rats were placed in restraining cages (W 80 mm × L 300 mm × H 150 mm, Yamanaka Chemical Ind., Ltd. Osaka, Japan). Intravesical pressure was kept constant by connecting a bladder catheter to a saline reservoir and a pressure transducer via three-way stopcocks. The reservoir was mounted on a metered vertical pole for controlled height adjustment. Intravesical pressure was abruptly increased to 20, 40 and 60 cm H_2O by elevating the reservoir and maintained at each pressure level for 2 min (Yoshizawa et al., 2015). Between pressure elevations, the reservoir was returned to 0 cm H_2O for 2 min. The MAP was electronically averaged from pressure values measured for 2 min before and during bladder distention using Chart software (AD Instruments, Colorado Springs, CO) (Fig. 1-B). Using a faster scale of arterial pressure tracing, heart beats were also counted for 10 s and the MHR was averaged for 1 min (Fig. 1-C). Arterial pressure and heart rate responses are shown as ΔMAP and ΔMHR by the difference of mean values measured before and during bladder distention.

2.3. Local suppression of NGF in the bladder after SCI

Cationic liposomes composed of DOTAP were made by the thin film hydration method (Fraser et al., 2003), during which a lipid film is hydrated with nuclease-free water to a final lipid concentration of 7 mM. The NGF antisense oligonucleotide (5′GCCCGAGACGCCTCCCGA3′) was dispersed in nuclease-free water at a concentration of 12 μM, and then conjugated with liposomes by incubating the two entities together at room temperature for 30 min. The molar ratio of NGF antisense oligonucleotide to lipid in the liposomal complex was 1:10. Seven weeks after SCI the rats were divided into 3 groups; (1) vehicle (saline) (n = 6), (2) liposome-only (n = 8) and (3) liposome-NGF antisense (n = 8) treated groups because 2 out 8 vehicle-treated SCI rats died