Journal of Pharmacological and Toxicological Methods 79 (2016) 7–14

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

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

Swing time ratio, a new parameter of gait disturbance, for the evaluation of the severity of neuropathic pain in a rat model of partial sciatic nerve ligation☆

Kenshiro Matsuda [a], Kensuke Orito [d], Yosuke Amagai [b], Hyosun Jang [a], Hiroshi Matsuda [a,b], Akane Tanaka [a,c,*]

[a] Cooperative Major in Advanced Health Science, Graduate School of Bio-Applications and System Engineering, Japan
[b] Laboratory of Veterinary Molecular Pathology and Therapeutics, Division of Animal Life Science, Institute of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan
[c] Laboratory of Comparative Animal Medicine, Division of Animal Life Science, Institute of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan
[d] Laboratory of Physiology II, Department of Veterinary Medicine, School of Veterinary Medicine, Azabu University, Kanagawa, Japan

ARTICLE INFO

Article history:
Received 6 October 2015
Received in revised form 17 December 2015
Accepted 23 December 2015
Available online 29 December 2015

Keywords:
Animal model
Gait disturbance
Swing time ratio
GAIT® system
Neuropathic pain
Partial sciatic nerve ligation
Pregabalin

ABSTRACT

Introduction: Dynamic weight bearing tests are used to evaluate the chronic pain severity in animal models of nociceptive pain (such as osteoarthritis); however, common tests frequently fail to collect the characteristics of neuropathic pain such as allodynia, because surgical intervention which is sometimes required to establish the models causes both nociceptive and neuropathic pain.
Methods: In this study, we used rats with partial sciatic nerve ligation (PSL) as the neuropathic and chronic pain model. To assess the severity of pain by gait disturbance, we applied automatic analysis on walking function using the GAIT® system. The system employs a novel index of abnormal step cycles, the swing time ratio (STR), of laboratory animals. Data were compared to those obtained with conventional tests, including a von Frey test and a hot plate test. Finally, we analyzed recovery of walking function after single or repeated administration of pregabalin.
Results: By using rats with PSL, we confirmed that results obtained by the GAIT® system were comparable to those obtained by both von Frey tests and hot plate tests. Single administration of pregabalin transiently improved STR, on the other hand, repeated pregabalin treatment showed lasting STR recovery.
Discussion: STR is sensitive to claudication of rats with PSL, providing a new scale to evaluate neuropathic pain in addition to conventional tests. Moreover, STR analysis enables us to evaluate walking function of animal models after neuropathic injury, which is quite important to judge the effectiveness of new treatments and analgesics.

1. Introduction

Neuropathic pain refers to pain that originates from pathological abnormalities of the central or peripheral nervous system (Loeser & Treede, 2008). For example, herpes zoster (Dworkin et al., 2008), diabetes (Ziegler, 2008a, 2008b), and cancer (Grond et al., 1999) can cause neuropathic pain, which is characterized by spontaneous burning pain accompanied by allodynia and hyperalgesia (Baron, Binder, & Wasner, 2010). Furthermore, neuropathic pain may be induced by physical injuries of the nervous system, such as surgery- and traffic accident-related injuries (Laird, Colvin, & Fallon, 2008). Conventional analgesics such as non-steroidal anti-inflammatory drugs (e.g. indomethacin) and narcotic analgesics (e.g. morphine) have limited therapeutic value for treating painful neuropathy (Galer, 1995; Ziegler, 2008a, 2008b). Recent studies have shown that pregabalin, the alpha$_2$-delta ligand of voltage-gated calcium channels, induces an anti-hyperalgesic effect in neuropathic pain, and suppresses static and dynamic allodynia through the inhibition of glutamate release in the spine (Gustafsson & Sandin, 2009; Kumar, Laferriere, Yu, Leavitt, & Coderre, 2010; Siddall et al., 2006).

To study the pathophysiological mechanisms underlying neuropathic pain, several animal models with clinical pain syndromes have been developed in the past decades (Wallace, 2001). Partial sciatic ligation (PSL), chronic constriction injury, spinal nerve ligation (SNL), and spared nerve injury are widely known surgical methods for inducing neuropathic pain in rodents (Seltzer, Dubner, & Shir, 1990; Wang & Wang, 2003). Although there are differences between these models, they all produce the behavioral signs of neuropathic pain, such as mechanical and thermal hypersensitivities (Lee, Yoon, Chung, & Chung, 1998). One of these models, the PSL-induced neuropathic pain model, promotes long-lasting neuropathic dysfunction (Seltzer et al.,

☆ Disclosures: This work was partially supported by a joint research fund from Noveltec Inc., (Kobe, Japan) (Grant No. 25-731).
* Corresponding author at: Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan.
E-mail address: akane@cc.tuat.ac.jp (A. Tanaka).

http://dx.doi.org/10.1016/j.vascn.2015.12.004
1056-8719/

1990). Therefore, PSL is widely used to unravel the pathogenesis of neuropathic pain and to evaluate the efficacy of potential drugs to alleviate pain (Shir et al., 2001; Taylor et al., 2007).

Assessment of nociceptive thresholds is useful for evaluating pain severity and therapeutic effects of analgesics in chronic pain. In patients, a standardized psychophysical technique has been employed to test the nociceptive afferent systems (quantitative sensory testing) and to evaluate hypersensitivity to pain (Freeman, Baron, Bouhassira, Cabrera, & Emir, 2014; Shy et al., 2014). In rodent models of chronic pain, hind paw withdrawal thresholds are evaluated using the von Frey test and the hot plate test (Abbadie et al., 2003; Lambert, Mallos, & Zagami, 2009; Shir et al., 2001). Existing studies quantify one particular aspect of sensory functions, and therefore, the mechanisms underlying neuropathic pain are poorly understood. Responses are not always well defined, and their interpretation may vary between estimators (Kim, Yoon, & Chung, 1997; Lambert et al., 2009). Measurement of dynamic weight bearing, which has been developed in the past decade, is a sensitive indicator of chronic pain in rodents (Mogil & Crager, 2004). In arthritis, dynamic weight bearing is more relevant clinically than static weight bearing, because walking aggravates pain in animals with arthritis (Clarke, Heitmeyer, Smith, & Taiwo, 1997). Additionally, the measurement of dynamic weight bearing is useful for evaluating the severity of arthritis and acute inflammation in animal models (Amagai et al., 2013). On the other hand, the establishment of animal models with neuropathic pain requires surgical intervention, which induces both nociceptive and neuropathic pain. Furthermore, in many popular techniques, animals must be restrained (Pitcher, Ritchie, & Henry, 1999) or are constrained into an unusual standing position on force plates (Min et al., 2001), indicating that researchers measure both nociceptive and neuropathic pain.

Recently, the GAIT® system, a novel automatic gait analyzer, has been developed to quantify gait disturbances in animal models of unilateral disorders such as osteoarthritis, intermittent claudication, and acute inflammation (Amagai et al., 2013; Okamoto et al., 2011; Orito et al., 2007). Swing time ratio (STR), which reflects walking function, is detected within each step cycle for 2 min by using the GAIT® system (Okamoto et al., 2011). In the present study, we applied the GAIT® system on a rat model of unilateral PSL for evaluating whether neuropathic pain could be accurately determined.

2. Methods

2.1. Animals

Adult male Wistar/ST rats (SLC Inc., Tokyo, Japan) aged over 15 weeks and weighing 403–581 g were used in all experiments. Animals were kept in a clear acrylic cage and had free access to standard chow and water. Temperature and humidity of the animal room were 22 ± 4 °C and 40 ± 15%, respectively. The animal room was maintained on a 12:12-h light–dark cycle. All animal experiments complied with the guidelines of University Animal Care and Use Committee of the Tokyo University of Agriculture and Technology, as well as with the guidelines of Science Council of Japan for the use of laboratory animals. All animal experiments in the present study were approved by the University Animal Care and Use Committee at Tokyo University of Agriculture and Technology. For the present study, the training was performed on 41 rats, 38 rats that showed abnormalities appeared in PSL were used for measurements.

2.2. Surgical procedure of PSL

According to a previous study (Seltzer et al., 1990), we used the rat model of PSL-induced neuropathic pain. Briefly, we anesthetized the animals with isoflurane (2–3%) inhalation (Small Animal Anesthetizer and Small Animal Ventilator, Muromachikikai Co., Ltd.; Tokyo, Japan), and the right sciatic nerve of each rat was exposed at high-thigh level by the ablation of the femur muscle (Supplementary Fig. 1A − D). The sciatic nerve was detached from the surrounding connective tissues using a honed (no. 5) jeweler's forceps (Supplementary Fig. 1E), and it was ligated at the half thickness of the nerve with 100 g tension using an 8–0 silicon-treated silk suture (Alfresa Pharma Co., Ltd.; Osaka, Japan). In rats subjected to the sham surgery, the nerve was left intact. The wound was closed with the same stitches after both sham and PSL operations (Supplementary Fig. 1F − G; 5–0 absorbable surgical sutures were used for the closure of subcutaneous tissues, and 3–0 cotton sutures were used for the closure of the skin). Both general conditions and activity of operated rats were assessed everyday by 2 different veterinarians visually. We confirmed the establishment of PSL by the visual check, hot plate test, and von Frey test before the application of GAIT® analysis.

2.3. Gait analysis

Gait analysis was performed as previously described (Okamoto et al., 2011) with slight modifications. Briefly, for 2 weeks, rats were trained to keep walking for 2 min in the acrylic wheel (400 mm diameter) of the GAIT® system, which revolved at 4.0–6.0 rpm (Orito et al., 2007). On the day of measurement, each rat was allowed to walk in the wheel at 4.0 rpm for 1 min. Then a gait movie was captured for 2 min by using Light Capture Version 1.00 (I-O data Device Inc., Ishikawa, Japan) (Min et al., 2001; Orito et al., 2007). In this study, we used STR which can be applied for rodents with both spontaneous and experimentally induced injury in hind limbs to evaluate walking function of rats. STR was calculated using the following formula: STR = (swing time of the normal hind limb) / (swing time of the painful hind limb). As the grounding hind limb bears dynamic weight during the swing, swing time of the painful hind limb is prolonged to avoid pain, which leads to the decrease of STR. According to the previous study (Okamoto et al., 2011), we performed 2-min measurement for the rat model of neuropathic pain. Number of step cycles and STR were automatically calculated by the GAIT® software based on a 2-min interval for each rat. To avoid data variation by fatigue of PSL rats and to evaluate quick assessment of gait disturbance, we selected one 2-min measurement in the current study.

2.4. von Frey test

Animals were habituated to the test cages for 15 min every day for 2 weeks before the experiment, and for 10 min before each measurement (Ferreira-Gomes, Adaes, & Castro-Lopes, 2008). The test was carried out before the surgery on day 3, and every week after the surgery for 5 weeks. According to the manufacturer's instruction (Ferreira-Gomes et al., 2008; Taylor et al., 2007), paw withdrawal threshold (PWT) was assessed as an index of mechanical allodynia by using the Dynamic Plantar Aesthesiometer (Ugo Basile Inc., Gemonio, Italy), which is an automated instrument for the von Frey test with a slight modification. Briefly, PWT was evaluated after applying an increasing force (continuous increase from 0 to 40 g in 40 s) to the plantar surface of the hind paw of each rat (Taylor et al., 2007). The touch was initiated when the rat was standing still on its extremities.

2.5. Unilateral hot plate test

Habituation was carried out using the same method as for the von Frey test. The unilateral hot plate test was used to assess paw withdrawal latency (PWL) as an index of static thermal allodynia in the hind paws of rats (Menéndez, Lastra, Hidalgo, & Baamonde, 2002). A hot/cold plate (Ugo Basile Inc., Gemonio, Italy) was maintained at 52.5 °C ± 0.2 °C with a cutoff time of 40 s, and the elapsed time between placement and withdrawal of the hind paw was measured for each rat.