**Pulse Diode Laser Irradiation (830 nm) of Lumbosacral Spinal Roots Diminished Hyperreflexia-Induced by Acetic Acid or Prostaglandin E2 Infusion in Rat Urinary Bladder**

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**Objectives:** Low power diode laser (830 nm) irradiation is a useful analgesic tool in superficial pain. Pulse laser irradiation allows us to increase the laser power because the non-irradiation time reduces heating effects and/or direct tissue damage at the irradiation area. This new irradiation device using pulse laser was applied to the dorsal skin to investigate the effects on the micturition reflex in the rat by targeting underlying sacral spinal roots.

**Methods:** Vesical pressure measurement during the continuous infusion of the urinary bladder with saline, acetic acid (AA, 0.1%) or prostaglandin E2 (PGE2, 10^{-5} M) were performed in un-anesthetized rats. Multi-unit recording from bladder afferent nerves preformed under urethane anesthesia. Laser irradiation, either continuously at 1 W or in 10 W-pulse mode, was delivered at 830 nm from 1.5 cm above the skin at the lumbosacral joint.

**Results:** During continuous saline infusion to the urinary bladder, neither continuous (1 W) nor pulse (10 W) laser irradiation altered the intercontraction interval and nerve firing during distention of the bladder. Pulse laser, but not continuous laser irradiation, increased the intercontraction interval with AA or PGE2 infusion and diminished nerve firing during distention of the bladder with AA or PGE2 infusion.

**Conclusion:** These data indicate that pulse laser could diminish inflammation related nerve firing from the bladder. Since this laser irradiation did not affect the normal bladder distention elicited nerve firing, it appears capable of reducing urgency sensation without loss of the basic micturition reflex.

**Key words** afferent nerve discharges, Ga-Al-As diode laser, micturition reflex

1. **INTRODUCTION**

Sensory signals during storage of the urine in the bladder are important for the initiation and completion of micturition.1–4 Excitatory inputs from the urinary bladder are mediated through the pelvic nerves (parasympathetic) and enter the spinal cord through the sacral spinal roots. Urgency sensation for micturition1,3–6 and pain7–9 sensation have common excitatory inputs because both are triggered by tissue damage, nerve injury, and inflammation. In addition, urgency sensation and pain are both mediated by small myelinated and unmyelinated sensory fibers, whereas somatic touch sensation is mediated by large myelinated fibers.1–3,7–9

Intravesical infusion of acetic acid (AA) or capsaicin in rats decreased inter-contraction interval (ICI), decreased bladder capacity, and increased afferent nerve activity during urine storage.10,11 Our recent study demonstrated that muscarinic receptors and transient receptor potential V1 (TRPV1) channel receptors are present in the urothelium and that administration of acetic acid, or capsaicin, opens the TRPV1 channel and released ATP in vivo and in vitro.12,13 In addition, intravesical infusion of prostaglandin E2 (PGE2) decreased ICI and increased afferent nerve activity.10,11,14,15 An EP1 receptor antagonist abolished the PGE2-mediated effect,10,15 and some studies showed that EP3 and EP4 receptors might play a role on the micturition reflex.16,17 which indicates that PGE2 might be important for afferent signals from the bladder. Of course, PGE2 released at the inflammation area and in tissue or cell damage causes acidosis. These findings suggest that unmyelinated C-fiber sensory nerve activity during the pathological conditions involving inflammation must be involved in overactive bladder.1,10

Diode laser irradiation (830 nm) has been used for analgesia in the past two decades.18–22 When laser irradiation is used on skin or muscles, pain sensation decreases without altering the normal skin sensation (i.e. touch)
because the laser selectively decreases C-fiber afferent activity in peripheral nerves.23–25 One issue remaining to be resolved, however, is that diode laser light is not transmitted deeply after penetrating the skin, hence this method of analgesia is limited to skin and superficial tissues. A higher laser power could transmit its energy more deeply, but high-energy irradiation causes skin and superficial tissue damage.

In the present study we used a pulse laser device which transmitted laser energy for 10% of the irradiation time.18 This reduces the total energy when compared with the continuous irradiation devices previously used, and the tissue is prevented from damage and local temperature increase by the no-irradiation periods. These two benefits allowed us to increase the power of the laser device, so that we could deliver equal irradiation energy with lower power continuous irradiation. For example, total energy can be reduced by a factor of 10 by reducing irradiation time to 10% compared with 100% continuous irradiation.

Since the lumbosacral spinal roots are relatively deep structures, pulse laser irradiation should be a good method to deliver reasonable energy to these deep nerves without damage to the skin and superficial tissues. We hypothesized that pulse diode laser irradiation at the lumbosacral nerve roots would diminish pathological C-fiber activity caused by acid stimulation or inflammation. Verification of this hypothesis would suggest that this form of laser might be a new tool for treatment of urgency sensation elicited by the peripheral sensory system.

2. METHODS

2.1. Materials

Wistar rats were used (10–20 weeks old, n = 26, female). Protocols for these experiments were approved by the Animal Research Committee at Akita University.

2.2. Continuous cystometry

Detailed methods have been published previously.10,11,15 Under halothane anesthesia, an intravesical catheter (PE-50) was inserted through the bladder dome (n = 16). After surgery, each rat was placed in a Ballman restraining cage and allowed to recover from anesthesia for few hours. Intravesical pressure was digitized with an AD converter and recorded with Power Lab system (ver. 5.0, AD Instruments, NSW, Australia). The experiment was performed with continuous infusion of saline, AA (0.1%), or PGE2 (10−5 M, Sigma, St. Louis USA) at a constant speed (0.04 mL/min). We previously determined the adequate PGE2 dose (10−5 M) for eliciting neuronal discharges in the C-fiber afferents from the urinary bladder without causing smooth muscle contractions.10

2.3. Neuronal discharge recording

In this experiment, rats were anesthetized with intraperitoneally administered urethane (0.75–0.9 g/kg, n = 10). Recording methods and the conditions in which animals were kept have been published previously.12 A polyethylene catheter (PE-50) was inserted into the jugular vein for drug administration. The trachea was also cannulated with a larger catheter (PE-200). A double-lumen catheter was inserted into the bladder through the urethra for infusion and recording of intravesical pressure. The lumbosacral spinal cord was exposed by laminectomy and covered with mineral oil, after which a fine filament of the L6 dorsal root was selected. Neuronal discharge was recorded with silver-chloride electrodes using an extracellular amplifier (DPA-100E, Diamedical, Tokyo, Japan). The spikes were selected with a window discriminator and the number of spikes per second was counted with a spike counter (DS-607, Diamedical). Intravesical pressure, discharge, and the number of spikes were digitized with an AD converter and recorded with Power Lab system (ver. 5.0, AD Instruments). The bladder was distended isotonically (to 30 cmH2O) for 2 min by raising the height of the reservoir tank. This stimulation was repeated every 10 min.

2.4. Laser irradiation

This was delivered at 830 nm to a round area (1.5 cm2) located 1.5 cm above the skin at the lumbosacral joint. Irradiation was performed either continuously at 1 W or in 10 W-pulse mode (10 ms on – 180 ms off). Body and skin temperatures were monitored before and after irradiation. Because the effect of laser irradiation depends on the energy delivered, we selected 0.67 W/cm2 for continuous irradiation (1 W output, 3 min) and 6.7 W/cm2 for pulse irradiation (20 ms on – 180 ms off, 10 W output, 3 min). During the experiments, the room temperature was maintained between 23 and 25°C and the humidity was maintained between 50 and 60%.

2.5. Histology and statistics

After the physiological experiments, the animals were perfused with physiological saline followed by 4% paraformaldehyde. The irradiated skin and deep connective tissue were resected and processed for histological analysis. Thin cryostat sections (20 μm) were mounted on glass slides and stained with hematoxylin-eosin. Tissue sections were examined microscopically. Data are shown as mean ± standard error and as percentage of pre-laser values, and statistical comparisons were performed using Student’s t-test. P < 0.05 indicated statistical significance.

3. RESULTS

When continuous cystometry (CMG) was performed with saline infusion, average ICI was 510.6 ± 52.1 sec and maximum voiding pressure (MVP) was 32.1 ± 2.7 cmH2O (Fig. 1). After continuous laser irradiation (1 W) to the lumbosacral area, ICI was 500.4 ± 80 sec (98%) and MVP was 32.6 ± 2.9 cmH2O (101%). After pulse laser irradiation (10 W), ICI was 480.1 ± 40 sec (95%) and MVP was 31.7 ± 4.1 cmH2O (97%). Hence, neither type of irradiation altered the ICI or MVP significantly (Fig. 1).

When CMG was performed with AA solution (0.1%), ICI was shortened from 496.1 ± 38 sec to 298.4 ±
Fig. 1 Effect of laser irradiation on continuous cystometrogram. (A) Continuous laser irradiation (1 W) for 3 min did not alter the interval of micturition reflex or maximum voiding pressure. (B) Pulse laser irradiation (10 W, 10% irradiation time) for 3 min also did not alter the micturition pattern.

38 sec (60%, \( P < 0.05 \)), but MVP was not altered (31.6 ± 2.1 cmH₂O to 27.8 ± 4.5 cmH₂O, Fig. 2). After continuous laser irradiation (1 W), both parameters remained unchanged; ICI was 310.4 ± 39.4 sec and MVP was 27.8 ± 4.5 cmH₂O. After pulse laser irradiation (10 W), ICI was significantly prolonged to 451.4 ± 65.9 sec (150%, \( P < 0.05 \)), but MVP remained similar at 28.3 ± 5.3 cmH₂O (Figs. 2, 3).

When CMG was performed with PGE₂ solution (\( 10^{-5} \) M), ICI was shortened from 503.1 ± 28.1 sec to 361.5 ± 39.4 sec (58%, \( P < 0.05 \)), but MVP was not altered (30.9 ± 4.6 cmH₂O to 28.5 ± 4.5 cmH₂O, Fig. 4). After

Fig. 2 Effect of laser irradiation on acetic acid infused continuous cystometrogram. (A) Acetic acid infusion shortened the interval of micturition reflex. (B) Pulse laser irradiation for 3 min prolonged the interval of micturition reflex.
continuous laser irradiation (1 W), both parameters remained unchanged; ICI was $384.9 \pm 40.6$ sec and MVP was $29.9 \pm 4.5$ cmH$_2$O. After pulse laser irradiation (10 W), ICI was significantly prolonged to $537.4 \pm 75.1$ sec (147%, $P < 0.05$), but MVP remained similar at $34.1 \pm 3.9$ cmH$_2$O (Figs 4, 5).

After continuous laser irradiation (1 W), body temperature increased by $0.1 \pm 0.1^\circ$C and skin temperature at the irradiated site increased by $0.15 \pm 0.1^\circ$C. After pulse laser irradiation (10 W), body temperature increased $0.12 \pm 0.1^\circ$C and skin temperature at the irradiated site increased by $0.23 \pm 0.1^\circ$C. These temperature changes were not statistically significant. Histological examination of the tissue revealed no edema or extravasated red blood cells in the irradiated skin.

In multi-unit recordings of bladder afferent nerve activity, neuronal discharge was observed immediately after bladder filling and increased to a maximum (plateau) within 30 sec. After 2 min of filling (30 cmH$_2$O), vesical pressure returned to normal levels and the discharge

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*[Fig. 3]* Bar graph shows acetic acid infusion (AA) shortened the interval of micturition reflex. Averaged maximum voiding pressure (MVP) was showed at bottom of the figure. AA did not alter the MVP. [Fig. 4] Line graph shows two types of laser irradiations effect of the interval of micturition reflex. Note pulse laser irradiation significantly increased the interval of micturition reflex. Averaged MVP was showed at bottom of the figure and either laser irradiations did not alter the MVP.

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*[Fig. 4]* Effect of laser irradiation on prostaglandin E$_2$ (PGE$_2$) infused continuous cystometrogram. (A) PGE$_2$ infusion shortened the interval of micturition reflex. (B) Pulse laser irradiation for 3 min prolonged the interval of micturition reflex.
diminished abruptly. The total number of spikes during bladder distention was reproducible in each rat (96.9 ± 3.4% of control values, n = 4, Fig. 6). The maximum number of spikes (10–14 counts/sec) was also reproducible (104.7 ± 7.1%, n = 4).

In saline-infused rats, neither type of laser irradiation (1 W continuous or 10 W pulse) altered the distension-elicted discharge (101 ± 4.1% of control or 103 ± 2.1%, respectively). Acetic acid infusion increased the total number of spikes to 155 ± 29.4% of the control values. Continuous laser irradiation (1 W) did not alter the number of spikes/sec (106 ± 10% of control) whereas pulse laser irradiation (10 W) diminished distension-related nerve discharges (25 ± 19% of control, P < 0.05, Fig. 6).

4. DISCUSSION

In the present study, we have shown that pulse laser (10 W), but not continuous laser (1 W) irradiation, increased the ICI with AA or PGE2 infusion to the urinary bladder.
bladder and diminished nerve firing during distention of the bladder with AA or PGE2 infusion, whereas neither continuous nor pulse laser irradiation altered the ICI and nerve firing during distention of the bladder during continuous saline infusion.

Filling the bladder with urine activates low-threshold (less than 10 cmH2O) stretch receptors, and the afferent signals are transmitted by myelinated fibers of pelvic nerves to the sacral spinal cord where they activate the micturition reflex. In somatic nerves, touch sensation from the skin is also carried by myelinated fibers. Previous studies have demonstrated that laser irradiation of isolated dorsal roots does not alter these myelinated fiber signals. However, it selectively diminishes action potentials conducted by unmyelinated fibers and thereby reduces inflammation and pain signals. Thus activation of myelinated afferent fibers by saline infusion of the urinary bladder should not be sensitive to laser irradiation. It is therefore reasonable that both continuous and pulse laser irradiation did not alter the ICI or the MVP during saline infusion in the present study.

Intravesical infusion of AA could activate acid-sensing ion channels in the urothelium, whereas capsaicin and PGE2 infusion could activate TRPV1 channels and EP1 receptors, respectively. Activation of these channels and receptors in the urothelium released ATP and activated the primary afferent terminals in the urinary bladder. Previous studies reveal that AA or PGE2 infusion of the bladder increased the number of unmyelinated fiber afferent discharges and lowered the threshold pressure. Hence, laser irradiation in this experiment diminished discharges following AA or PGE2 infusion.

In 10 W pulse laser irradiation, energy is transmitted for 20 ms followed by a 180 ms rest period. Since irradiation energy was calculated as 10% of total irradiation time, total energy must be approximately equal to that delivered by continuous 1 W irradiation. The higher power (10 W) laser energy is easily transmitted to the tissue, which could increase the local temperature and result in tissue damage. In this experiment, however, the skin temperature increase was only 0.15 ± 0.1°C (with 1 W) or 0.23 ± 0.1°C (with 10 W). Histological examination of the tissue revealed no edema or extravasated red blood cells in the irradiated skin. These findings indicate that pulse laser irradiation did not change the skin temperature or cause acute inflammation and skin damage.

Following AA or PGE2 infusion, ICI was not altered by continuous laser irradiation but was prolonged by pulse laser irradiation. This discrepancy indicates that the spinal nerves mediating the bladder afferent signals were located relatively deeply. It is likely 1 W laser irradiation had inadequate energy to reach to these nerves but that 10 W laser irradiation was sufficient to do so.

The mechanism of laser irradiation on peripheral nerves transmitting pain remains unclear. Laser appears to diminish unmyelinated fiber activity but not myelinated-fiber activity. Moreover, recent studies have demonstrated that it decreases inflammation related to IL1β mRNA and PGE2 mRNA and diminishes PGE2-induced and TRPV1-induced signals. Hence, laser irradiation might act on membrane proteins within nerve fibers. Neuronal hyperpolarization occurring after laser irradiation indicates that laser irradiation diminishes pain-related signals at the nerve fibers.

In summary, laser irradiation from the dorsal skin to the lumbosacral spinal nerves was difficult because these nerves are anatomically deep. Nonetheless, the present pulse laser irradiation significantly changed ICI after the urinary bladder was infused with AA or PGE2. It did not alter ICI after saline infusion. It is likely that AA or PGE2 infusion elicits abnormal sensation from the bladder which is mediated by C-fibers, and that pulse laser irradiation diminishes this type of neural activity. Our previous data suggest that laser irradiation to peripheral nerves selectively inhibits C-fiber mediated pain signals. Thus pulse laser irradiation must act on spinal roots carrying bladder afferent nerve activity and selectively inhibit pain- and inflammation-related signals. We therefore suggest pulse laser irradiation as a possible treatment for patients with overactive bladder.

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