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# Ga-Al-As LASER IRRADIATION INHIBITS NEURONAL ACTIVITY ASSOCIATED WITH INFLAMMATION

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**ABSTRACT:**

A Ga-Al-As diode system that produces low-energy red light (830 nm, 40 mW) has been used for the treatment of many kinds of pain. The mechanism of action of this new laser irradiation for analgesia was studied in anesthetized rats. The effect of laser irradiation of the saphenous nerve was studied by recording neuronal activity at the L4 dorsal root filaments after the injection of a chemical irritant, turpentine. Laser irradiation inhibited both the asynchronous firing by that was induced by turpentine and increased part of the slow components of the action potentials. Thus, the laser irradiation selectively inhibited nociceptive signals at peripheral nerves.

KEY WORDS: acute inflammation, antinociception, axonal conduction, pain, saphenous nerve

## INTRODUCTION:

Laser irradiation has been used for treatment of many kinds of pain, as well as hypersensitivity of the skin and/or the mucosa (1,2,3,4,5,6,7,8,9). Recent technologic advances have led to development of new, invisible red-light-producing laser known as the Ga-Al-As diode system (830 nm, 40-60 mW) during the past decade (1,5). This system produces stable, relatively high-energy

light. The peak wavelength is 830 nm which is poorly absorbed by tissue and has only a limited effect on hemoglobin in red blood cells (1). This device is now used for treatment of pain and hypersensitivity conditions instead of the original 630 nm low-energy red light from a He-Ne laser. Most beneficial effects have been observed when irradiation was directed at the area of abnormal sensation and its innervating nerves (1,2,3,4). Even though this laser irradiation has been widely used on patients, no systematic analysis of the pain-relief mechanism has been made in animal models of pathological conditions.

In a clinical setting, the 830 nm laser irradiation inhibits neither motor movement nor normal sensations (1,2,5,6). One possible effect of the laser is to selective inhibition of abnormal sensation. In general, faster-conducting fibers mediate touch and pressure sensory systems and slower-conducting fibers mediate nociceptive sensory systems. Electrophysiological experiments allow us to differentiate between the faster- and slower- conducting fibers, and we have attempted to demonstrate the effects of laser irradiation on signals due to inflammation. It has been demonstrated that injection of formalin, turpentine or BCG to the hind paw of the rat elicits neuronal discharges (10,11). Therefore we examined whether the laser irradiation could inhibit neuronal signals elicited by injection of chemical that causes a acute inflammation.

A relationship between the type of nerve fiber and the associated conduction velocity in L4 dorsal root ganglion cells in the rat was demonstrated recently (12). The L4 dorsal root receives sensory input from the paw via the saphenous nerve. In our experiments, we stimulated or injected the paw and recorded the activity from the L4 dorsal root, with laser irradiation of the saphenous nerve. Since the saphenous nerve is a purely sensory nerve fiber, the effect of irradiation is exclusively on the sensory nerve fibers. Preliminary data have been published elsewhere as an Abstract (13).

## MATERIALS AND METHODS:

### Electrophysiological Experiments:

25 adult male and female Wistar rats (body weight, 250-450 g) were used for the experiments. After anesthesia with urethane (1-1.2 g/kg, i.p.), a polyethylene tube (PE-240) was inserted into the trachea. Exposure of the lumbosacral spinal cord was accomplished through a dorsal mid-line incision. After opening the dura mater, the L4 dorsal root was dissected out for unit recording (Fig 1). The saphenous nerve was exposed between the knee and the ankle and dissected free from connective tissue and blood vessels for both electrical stimulation and laser irradiation (Fig 1). Both the exposed back space and hind paw were filled with paraffin oil at a temperature of 33-36 °C. A chemical irritant, turpentine (0.1 ml, s.c.) was injected into the paw. A silver-hook unipolar electrode was placed on the L4 dorsal root to record both the neuronal discharges and axonal volleys. Neuronal discharges were selected by window discrimination from the baseline and numbers of discharges per

second were counted with a spike counter (DSE-325P; Diamedical Co., Tokyo, Japan) and printed on a pen-recorder. Electrical stimulation of the saphenous nerve was achieved with a silver hook bipolar electrode and a short pulse (0.1-100 V, 0.01 ms duration; SEN3201; Nihonkohden, Tokyo, Japan). The frequency of stimulation was 1 Hz. The data were recorded with a preamplifier (Biophysical Amplifier AVB-10; Nihonkohden) and were displayed on an oscilloscope (VC-10; Nihonkohden). They were also averaged by a digital computer system (DPC-1200-AD, DPC-1200-PA; Diamedical Co. Ltd., Tokyo, Japan). Thirty-two individual responses were averaged by the computer in each case. The intensity of stimulation for production of the maximum size of each component of the response was maintained before and after the laser irradiation.

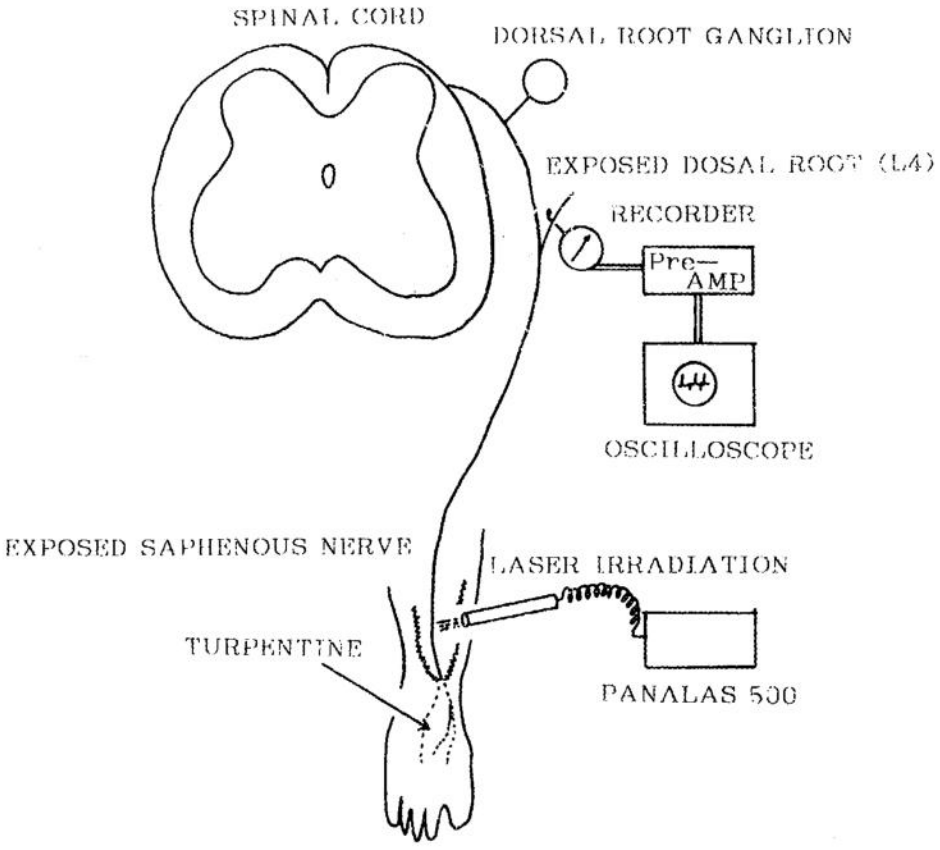


Fig 1. Schematic diagram showing the components of saphenous nerve, site of recording, turpentine injection and laser irradiation in the rat.

Diode laser irradiation:

Diode laser irradiation (830 nm, 40 mW, continuous wave; Panalas 500; Mochida Medical Instruments, Tokyo, Japan) to low leg skin or to the exposed saphenous nerve was applied 1 cm central to the electrical-stimulation electrode or 2 cm central to the site of injection of the chemical irritant, turpentine (Fig 1). Light at a wavelength of 830 nm was produced by a gallium-aluminum-arsenide (Ga-Al-As) laser and was transmitted through glass fibers (diameter, 2 mm) to the tissue. The dose at the focal point was estimated to be approximately 1 W/cm$^2$. The ends of glass fibers were located 0.5-1.5 mm away from the exposed nerve. Laser irradiation was applied continuously for 30, 60 or 180 sec.

Statistics:

Conduction velocity was calculated from the time and the distance between the stimulating and recording electrodes. All results are presented as means ± SD. Student's t-test was used to identify the statistical significance of the effect of laser irradiation on the maximum size of each component or the area under the curve of the computer-averaged axonal volley.

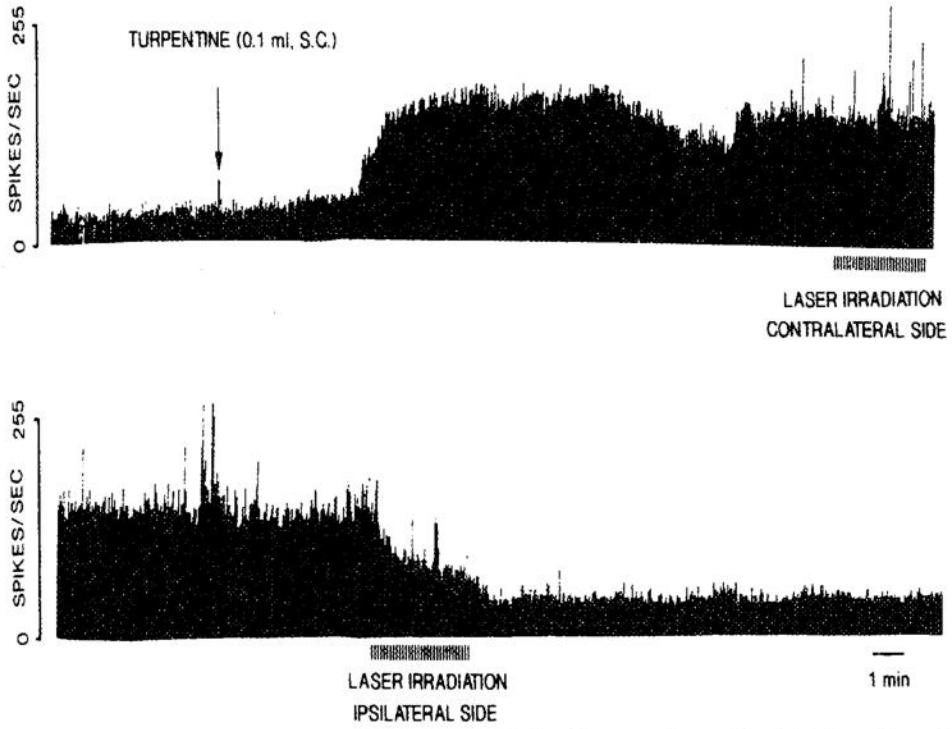


Fig. 2: Rate-meter recordings of neuronal discharges from the L4 dorsal root filament. The recordings continue from the top trace to the bottom trace. An arrow indicates the injection of turpentine into the paw. The first dashed bar (top trace, right side) indicates the laser irradiation (40 mW, 3 min, continuous wave) to the saphenous nerve contralateral to the site of the injection. The second dashed bar (bottom trace) indicates laser irradiation (same parameters) to the saphenous nerve (ipsilateral) to the injection.

**RESULTS:**

Turpentine induced discharges:

In 14 of 18 experiments, spontaneous neuronal discharges in the dorsal root filament were increased (average 278±60%) by injection of turpentine into paw skin on the ipsilateral side (0.1 ml, s.c., Fig 1). The discharges increased 3-15 min after injection and reached to maximum within 10-20 min, being maintained for 60-90 min.

The turpentine-induced neuronal discharges were inhibited by continuous laser irradiation for three minutes to low leg skin in 3 experiments and to the saphenous nerve in 11 of 14 experiments (Fig 2). The number of spikes per sec on returned to the control level during irradiation or immediately after irradiation (Fig 2). In three experiments, a second injection of turpentine to the paw 2-3 hours after the first injection again increased the discharges (average 201±42%).

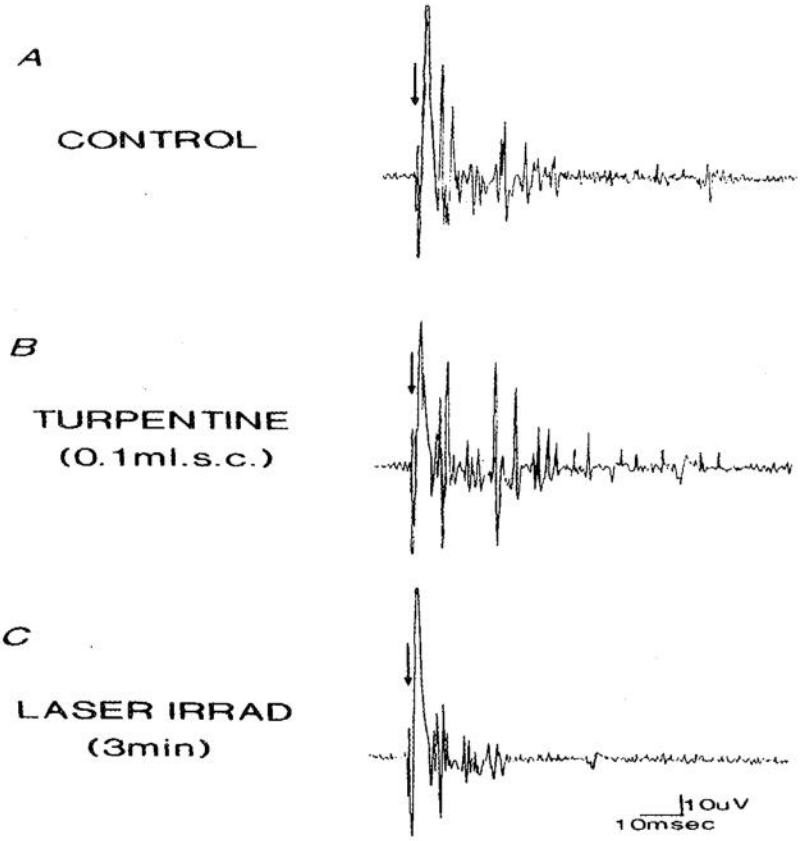


Fig. 3: The inhibitory effects of laser irradiation on the turpentine-induced slow-conduction part of axonal volleys from the dorsal root. Each record is a computer-generated average of 32 individual sweeps. Arrows indicate stimulus artifacts. (A) Control. (B) 3 min after injection of turpentine into the paw skin on the ipsilateral side. (C) 3 min after laser irradiation.

Compound action potentials

Electrical stimulation of the branch of the saphenous nerve that innervated the paw evoked an axonal volley on the ipsilateral side of the L4 dorsal root (N=17, Fig 3A). The latency for recorded components was 1.8-100 msec with a conduction distance of 60-70 mm and the range of threshold intensities was 0.5-80 V. In two experiments, the stimulating electrode was located in two different parts of the saphenous nerve for measurements of conduction velocity. Using the method of Lawson et al.(12) for estimating conduction velocity (CV), we separated fibers into four groups: Aα/β (CV>12 m/s); Aδ (CV=2-12 m/s); Aδ/C (CV=1.3-2 m/s) and C (CV<1.3 m/s) fibers. The measured latencies of axonal volleys for Aα/β fibers (threshold intensity (T)=0.5-2 V, N=12) was 1.8-5 ms; for Aδ fibers (T=1-6 V, N=17) it was 6-18 ms; for Aδ/C fibers (T=5-18 V, N=8) it was 19-36 ms, and for C fibers (T=19-80 V, N=5) it was 37-100 ms.

Injection of turpentine into the paw increased the contributions of the Aδ (CV=2-12 m/s), Aδ/C (CV=1.3-2 m/s) and C fiber components in the compound action potentials (Fig 3). By contrast, the Aα/β fiber components did not change. The average increased size of each components of the multi-compound action potential was 98.1±9.5%, 133±14.1%, 130.9±18.2% and 126±21.7% for Aα/β, Aδ, Aδ/C and C fibers, respectively (Fig 4). These increases for Aδ, Aδ/C and C fiber components were statistically significant. The increased sizes of these components were observed within 5 min after injection and a maximum was reached at 10-15 min, with maximum values persisting for 90 min. Hence, injection of turpentine appeared to increase unmasked slower components of action potentials. Newly appearing Aδ/C components were observed in four of nine experiments, and newly appearing C fiber components were observed in four out of twelve experiments.

The increases in the slower components of multi-compound action potentials (Aδ, Aδ/C and C fibers) caused by turpentine were eliminated by laser irradiation (Fig. 4). By contrast, the faster part of these action potentials (Aα/β fibers) was not affected (Fig 4). The inhibition of the slower components of action potentials was time-dependent. The extent following 30-sec irradiation was 108±29%, 116±26% and 106±39% from before the irradiation for Aδ, Aδ/C and C fibers, respectively. The extent following 60-sec irradiation was 102±31%, 94±21% and 94±43% for Aδ, Aδ/C and C fibers, respectively. The absolute values after 30-sec and 60-sec irradiation for each respective component were not significantly different from each other or from those of each respective component immediately after injection of turpentine. The extent following 180-sec irradiation was 97±13%, 85±26% and 86±30% for Aδ, Aδ/C and C fibers, respectively. Each component after 180-sec irradiation was significantly different from the corresponding component after injection of turpentine, but not significantly different from the corresponding component before injection of turpentine. The inhibition by laser irradiation was detectable immediately after the irradiation and persisted for more than 2 hours. After 2 hours, partial reversal of the effect was observed for 24%, 13% and 0% for Aδ, Aδ/C and C fibers components, respectively.

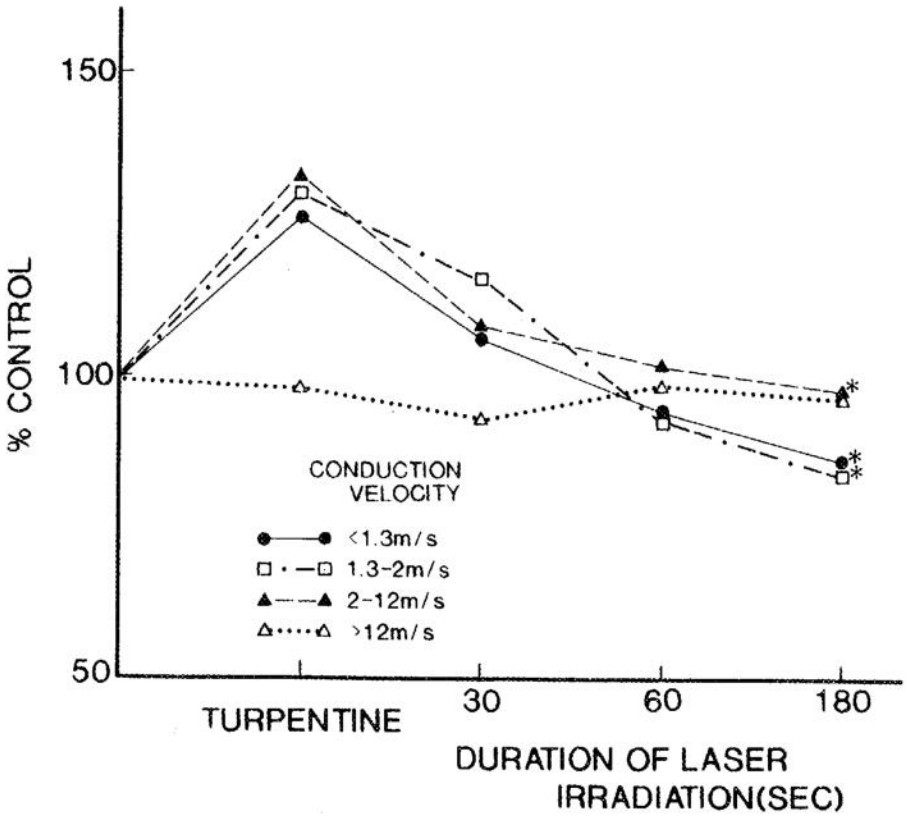


Fig. 4: Size of multi-unit action potentials after injection of turpentine and after laser irradiation (40 mW, continuous wave) for different times. Estimated conduction velocities were separated into four groups as indicated by different symbols. Each point is an average of 5 measurements. Note: the slower components of axonal volleys (CV<12 m/s) increased after injection of turpentine to the paw, and they returned to the control levels after 3 min laser irradiation. The fast components of axonal volleys were not altered by either turpentine or laser irradiation. * significantly different from the control, P<0.05.

**DISCUSSION:**

Acute inflammation induced by injection of turpentine to the paw produced new, spontaneous, neuronal activity in the ipsilateral L4 dorsal root filaments of the rat. Turpentine also increased the size of slower components of multi-compound action potentials elicited by stimulation of the saphenous nerve. Ga-Al-As laser irradiation both inhibited newly appearing, spontaneous, neuronal activity and increased the contribution of the slower components of the action potentials. These data suggest that such laser irradiation might selectively inhibit newly appearing neuronal activity that is elicited by acute inflammation.

Injection of turpentine produced new, asynchronous discharges, an indication that this chemical generated abnormal signals as a result of inflammation. Changes in conduction velocity due to turpentine might be attributable to an increase in excitability of nerves with slower conduction velocities. It appeared that Aδ, Aδ/C and C fiber components in multi-compound action potentials, which presumably include nociceptive responses, were involved in the turpentine-induced newly appearing asynchronous discharges.

The different effects of laser irradiation on action potentials of fast and slow conducting nerve were unexpected. Laser irradiation selectively inhibited the increased part of slow components of action potentials that were elicited by turpentine. However, this result fits with clinical data; laser irradiation alters abnormal sensations that include pain, but it does not alter responses related to touch and light pressure (1,2). Honmura et al. reported findings similar to our results (13). Laser irradiation did not alter the latency for heat-shock test in control groups. However after carrageenan had been injected into the paw, the laser irradiation decreased the latency to close to the control level.

In contrast of our direct inhibition of responses to the abnormal signals, irradiation with a He-Ne (630 nm, 0.3-1 mW) laser increases compound action potentials (15) and elicits evoked potentials from the somatosensory cortex (16). It has also been demonstrated He-Ne irradiation at acupuncture points causes analgesia (5,8,9,17). Analgesia by He-Ne laser irradiation is mediated by activation of acupuncture points and activation of the spinal or super spinal descending inhibitory system(9,17), as in the case of acupuncture (18). However, the present diode laser induced analgesia selectively by inhibiting nociceptive signals from peripheral receptors. Thus, this study clearly demonstrates that the anti-nociceptive effects of the He-Ne laser and the present diode laser are different.

The inhibitory effect of neuronal signals by focused laser irradiation could raise the possibility of nerve damage. Histological study demonstrated no degeneration (19) and damage on the plasma membrane and mitochondria (20). In our experiments, turpentine injection following laser irradiation produced asynchronous discharge suggested nerve fibers which sensitive to the nociceptive signals were not damaged (21).

Previously, very low power irradiation (0.07-1.56mW) to the nociceptive cells in hirudo medicinalis demonstrated that it did alter membrane potentials and their excitability (22). Higher power laser irradiation (40 mW) was shown to depolarize cells of the dorsal root ganglion in the rat (23). This result raised the possibility that inhibition of nerve conduction by laser irradiation might be caused by depolarization blockade. Our recent preliminary data suggest that lower energy (10-20 mW, 1 min, continuous irradiation) elicits asynchronous discharges (23).

## CONCLUSION:

Injection of turpentine into the paw in the rat produced asynchronous firing and increased the slow components of axonal conduction that mediate nociceptive signals during inflammation. Laser irradiation to the sensory nerve, namely, the saphenous nerve, inhibited both the asynchronous firing caused by turpentine and increased the size of slow components of the action potentials. Thus, laser irradiation selectively inhibited nociceptive signals without altering the normal somatosensory signals.

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## REFERENCES

1. Pontinen, P.J., Low Level Laser Therapy as a Medical Treatment Modality. Art Urpo Ltd: Tampere (Finland), pp11-211, 1992.

2. Nishida, N., Takigawa, C., Goda, Y., et al., Laser therapy for post therapeutic neuralgia. Pain Clinic, Vol.8, pp6-11, 1987.

3. Matsumoto, K., Funai, H., Wakabayashi, H., et al., Study on the treatment of hypersensitive dentine by Ga-Al-As laser diode. Journal of Japanese Conservative Dental, Vol.28, pp766-771, 1985.

4. Walker, J.B., Relief from chronic pain by low power laser irradiation. Neuroscience Letters, Vol.43, pp339-344, 1983.

5. Krotelinger, M., On the use of the laser in acupuncture. Acupuncture and Electro-Therapeutics Research: The International Journal, Vol 5, pp297-311, 1980.

6. Trelles, M.A. and Rotinen, S., He/Ne laser treatment of hemorrhoids. Acupuncture and Electro-Therapeutics Research: The International Journal, Vol 8, pp289-295, 1983.

7. Zalesskiy, V.N., Belousova, I.A. and Frolov, G.V., Laser-acupuncture reduces cigarette smoking. Acupuncture and Electro-Therapeutics Research: The International Journal, Vol 8, pp297-302, 1983.

8. Choi, J., Srikantha, K. and Wu, W., A comparison of electro-

acupuncture, transcutaneous electrical nerve stimulation and laser photo-biostimulation on pain relief and glucocorticoid excretion. Acupuncture and Electro-Therapeutics Research: The International Journal, Vol 11, pp45-51, 1986.

9. Kreczi, T. and Klingler, D., A comparison of laser acupuncture versus placebo in radicular and pseudoradicular pain syndromes as recorded by subjective responses of patients.Acupuncture and Electro-Therapeutics Research: The International Journal, Vol 11, pp207-216, 1986.

10. Rosa, M.D., Giroud, J.P., and Willoughby D.A., Studies of the mediators of acute inflammatory response induced in rats in different sites by carrageen and turpentine. Journal of Pathology, Vol.104, pp15-28 1971.

11. Dickenson, A.H. and Sullivan, A.F., Subcutaneous formalin-induced activity of dorsal horn neurons in the rat. Pain Vol.30, pp349-360, 1987.

12. Lawson, S.N. and Waddell, P.J., Soma neurofilament immunoreactivity is related to cell size and fiver conduction velocity in rat primary sensory neurons. Journal of Physiology (London), Vol.435, pp41-63, 1991.

13. Sato, T., Tsuchiya, K., Kawatani, M., et al., Diode laser irradiation inhibits neuronal activity elicited by chemical irritant in the rat. Japanese Journal of Anesthesiology, Vol.42, pp224, 1993.

14. Honmura, A., Yanase, M., Obata, J. et al.,Therapeutic effect of Ga-Al-As diode laser irradiation on experimentally induced inflammation in rats. Laser in Surgery and Medicine, Vol.12, pp441-449, 1992.

15. Rochkind, S., Nissan, M., Lubart, R. et al., The in vivo nerve response to direct low energy laser irradiation. Acta Neurochirurgica (Vienna), Vol.94, pp74-77, 1988.

16. Walker, J.B. and Akhanjee, L.K., Laser-induced somatosensory evoked potentials: Evidence of photosensitivity in peripheral nerves. Brain Research, Vol.344, pp281-285, 1985.

17. Ponnudurai, R., Zbuzek, V., Niu, H. and Wu, W., Laser photobistimulation-induced hypoalgesia in rats is not naloxone reversible.Acupuncture and Electro-Therapeutics Research: The International Journal, Vol 13, pp109-117, 1988.

18. Takeshige, C., Oka, K., Mizuno, T. et al., The acupuncture point and its connecting central pathway for producing acupuncture analgesia. Brain Research Bulletin, Vol.30, pp53-67 1993.

19. Maeda, T., Morphological demonstration of low reactive laser

therapeutic pain attenuation effect of the gallium aluminum arsenide diode laser. Laser Therapy, Vol.1, pp23-26, 1989.

20. Dimitriadis, V.K. and Liapis I.A., Effect of He-Ne laser on the midgut cells of dorsophia auraria larvae and its correlation with acupuncture. Acupuncture and Electro-Therapeutics Research: The International Journal, Vol 10, pp67-72, 1985.

21. Tsuchiya, K., Kawatani, M., Takeshige, C., et al., Laser irradiation abates neuronal responses to nociceptive stimulation of rat-paw skin. Brain Research Bulletin, Vol.34, pp369-374, 1994.

22. Lundeberg, T., Hode, L. and Zhou, J. Effect of low power laser irradiation on nociceptive cells in hirudo medicinalis. Acupuncture and Electro-Therapeutics Research: The International Journal, Vol 13, pp99-104, 1988.

23. Shimoyama, N., Iijima, K., Mizuguchi, T. et al,. Effects of low power laser irradiation on electrophysiological characteristics of dorsal root ganglion neurons of rats. Pain Research, Vol.6, pp111-115, 1991.

24. Yazawa, M., Kawatani, M., Tsuchiya, K. et al., Diode laser irradiation blocks the irritation discharge by depolarization. Japanese Journal of Anesthesiology, Vol.45, p229 1994.