Rehabilitation Practice and Science

Volume 43
Issue 4 *Taiwan Journal of Physical Medicine and Rehabilitation (TJPMR)*

Article 5

12-31-2015

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Recommended Citation

Lai, Yi-Hsuan; Tsai, He-Yu; Huang, Shih-Yang; Hsieh, Wei-Chi; Chen, Chien-Min; Hong, Chang-Zern; Hsu, Hung-Chih; and Chen, Kai-Hua (2015) "The Effect of Low Level Laser Therapy with Cluster Probe on Myofascial Trigger Spots in a Rabbit Model: A Case-Controlled Trial," *Rehabilitation Practice and Science*: Vol. 43: Iss. 4, Article 5.
DOI: https://doi.org/10.6315/2015.43(4)05
Available at: https://rps.researchcommons.org/journal/vol43/iss4/5

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Original Article

The Effect of Low Level Laser Therapy with Cluster Probe on Myofascial Trigger Spots in a Rabbit Model: A Case-Controlled Trial

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Objective: This study investigated the effect of cluster probe low level laser therapy (LLLT) with a continuous wavelength of 830 nm or 980 nm on the myofascial trigger spot (MTrS) in rabbit skeletal muscle, an animal model of myofascial pain syndrome (MPS).

Methods: Thirty-seven New Zealand rabbits with MTrSs in the thigh muscle were divided into three groups and were given placebo treatment (0 J/leg/day), 830 nm LLLT (6 J/leg/day), and 980 nm LLLT (6 J/leg/day), respectively, for 5 days. Electromyographic studies were used to evaluate the endplate noise (EPN) prevalence in each MTrS before treatment, on the first and fifth day after therapy.

Results: EPN prevalences decreased in all groups over time ($p<0.05$). However, there were no statistical differences among the three groups on the first or fifth day after therapy ($p>0.05$).

Conclusion: EPN in the rabbits' MTrSs in all three groups decreased on the first and fifth day after therapy but there were no statically significant differences among them. These results indicated that the cluster probe LLLT used in this study did not have a significantly better inhibitory effect on the rabbits' MTrSs than placebo therapy. (Tw J Phys Med Rehabil 2015; 43(4): 251 - 262)

Key Words: cluster probe, low level laser therapy, myofascial pain, rabbit, wavelength

INTRODUCTION

Low level laser therapy (LLLT) is the use of light waves typically in the 600–1000 nm wavelength spectrum to transfer energy to biological tissues for therapeutic outcomes.[1] For the past 30 years, it has been widely used for the clinical treatment of myofascial pain syndrome

Submitted date: 27 April 2016 Revised date: 27 June 2016 Accepted date: 11 July 2016

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(MPS), a condition caused by myofascial trigger points (MTrPs).[2-16] Numerous double-blind placebo-controlled studies had demonstrated that LLLT had positive therapeutic effects on pain relief, range of motion, muscle performance, and amelioration of disability,[2-6, 8-9, 13-16] but other studies reported no significant therapeutic effect of LLLT.[7, 10-12] This inconsistency might be related to external factors (such as characteristics of the skin, depth of the target site, and frequency of treatment) or internal factors (such as laser wavelength, use of continuous or pulsed laser light, and intensity of laser emission).[3, 17-20] In recent five years, different types of cluster probe LLLT have been developed: LLLT was reported to increase exercise performance and to decrease exercise-induced oxidative stress as well as the delay type of muscle soreness after exercise.[21-23] However, the effect of cluster probe LLLT on myofascial pain is still unknown.

To date, a drug- or injury-induced myofascial pain animal model has not been developed. The most acceptable myofascial pain animal model for studying MTrPs is based on a study by Hong and Torigoe conducted in 1994.[24] They found that in rabbit skeletal muscle, taut bands similar to those in human muscle could be identified by finger palpation. When a certain sensitive site in the palpable taut band was squeezed or compressed, the rabbit would express pain or discomfort with actions such as kicking or withdrawing, which were not observed when another site was similarly irritated. When such sensitive sites were stimulated mechanically with a blunt metal probe (snapping or tapping) or by a needle, local twitch responses (LTRs) could be observed. LTRs were elicited much more easily at this sensitive spot than at other sites in the same muscle. This hyperirritable spot was defined as a "myofascial trigger spot (MTrS)"and is similar to the human MTrPs. Rabbit localized twitch responses (R-LTRs) are similar to human LTRs with regard to both the characteristics of visible muscle twitching and EMG recording. Spontaneous electrical activity [i.e., endplate noise (EPN)] can be recorded from the minute locus of either an MTrS[25] or an MTrP.[26] This animal model has been very useful for studying the pathophysiology of MTrPs. By using this animal model, it was possible to evaluate the effects of LLLT in our previous studies.[27, 28]

This study was designed to investigate the effect of cluster probe LLLT on MTrSs and the effects of different continuous wavelengths of LLLT on the efficacy of treating MTrSs in a rabbit model of MPS by measuring the EPN prevalence.

METHODS

General design

This case-controlled study collected 37 New Zealand rabbits and divided them into three groups based on the treatment of MTrSs in both hind legs. The protocol was shown in Figure 1. Each rabbit received treatment on five consecutive days. The rabbits in the control group received five sham treatments, those in the 830 nm LLLT group received five treatments with a continuous 830nm LLLT, and those in the 980 nm LLLT group received five treatments with a continuous 980nm LLLT. EPN of the MTrS was assessed with an EMG needle at three time points: before treatment, after 1st treatment, and after 5th treatment. If a hind leg had no palpable taut band or if the baseline EPN prevalence measurement was too low (<24%), it was excluded from statistical analysis. To avoid subjecting the rabbits to painful sensations, the LLLT and EPN assessments were performed under general anesthesia. The investigator for the outcome measurement was blinded to the group allocation. The rabbits were administered with ketoprofen (3 mg/kg) for pain control and with cefazolin (250 mg/3 kg) for infection control each day. The study design and procedures were approved by the Committee on Animal Care and Use in our hospital (No. 2011112204). All rabbits were cared in the laboratory animal center, which has accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC, International).

Animal preparation

After general anesthesia, rabbits were weighed, and their both legs were shaved. The taut bands in the thigh muscles of both hind legs were identified and marked with an indelible marker on the skin by one of the investigators. After the skin of this region was incised, the taut band within the skeletal muscle was reconfirmed by the same investigator and the taut band was then encircled

with 3-0 nylon suture. This encircled area (0.5 × 0.5 cm^2) was used for LLLT and measurement of EPN.

LLLT

A continuous-wave gallium aluminum arsenide (GaAlAs) cluster low level laser device (Many Channels Laser Instrument, model TI-816-2D; Transverse Industries Corporation, New Taipei City, Taiwan; approved for medical use by the Food and Drug Administration, Ministry of Health and Welfare in Taiwan) with an output of either 830 or 980 nm was used. The laser device, which contained six laser diodes in a cluster probe (Figure 2), was different from that used in traditional LLLT. The cluster probe was placed lightly at 0.5 cm above the surface of the encircled taut band (Figure 3). The area covered by the cluster probe was 12.25 cm^2. The spot size of each diode was 0.01 cm^2 in the 830 nm group and 0.02 cm^2 in the 980 nm group; this was related to the different divergence angles of different wavelengths. Thus, the LLLT delivered energy not only to the taut band but also to the adjacent muscle. To avoid any decline in laser energy, the LLLT was performed with the rabbit under general anesthesia and with the muscle exposed during each session. In the 830 nm group, the power output was 180 mW and the irradiation time was 34s; in the 980 nm group, the power output was 300 mW and the irradiation time was 20s. The calculated energy was 6 J (power output, mW × irradiation time in seconds) in both experimental groups. In the control group, the power output of the laser device was adjusted to 0 and the irradiation time was 20s. The total laser energy in both experimental groups was the same (6 J/leg/session; total dose of 30 J over five sessions). Other parameters of the laser settings were summarized in Table 1. The laser application procedures performed for all the three groups were the same, except for the wavelength and laser energy of each experimental group.

Outcome measurement

The outcome measure was EPN prevalence, as determined by an EMG needle, at the following three time points: before treatment, after the 1st treatment (post-1st treatment), and after the 5th treatment (post-5th treatment). For measurement of EPN, an EMG machine (Galileo Nemus space channel EMG/NCV/EP system; EB Neuro, Italy) with monopolar EMG needle electrodes (37-mm, disposable, Teflon-coated, model: 902-DMF37-TP, VIASYS, Cardinal Healthcare, USA; the use of this model was approved for medical use by the Food and Drug Administration, Ministry of Health and Welfare in Taiwan) was used. The setting of the EMG machine included two EMG needles and one montage. The needle for recording EPN (called the "search needle") was connected to channel 1, and the control EMG needle was connected to channel 2. The search needle was inserted into the MTrS region and the control EMG needle was inserted into the non-taut band region in the muscle adjacent to the MTrS (Figure 3). The montage was completed by the application of a 2-cm diameter disc ground electrode to the ear lobe. The sensitivity was 50 μV/division, and the sweep was 10 ms/division.

EPN was measured as previously described[29] by an investigator who was blinded to group allocations. As with the LLLT, EPN was measured with the rabbit under general anesthesia and with the muscle exposed. The search needle was inserted into the region of the taut band (which was encircled by the nylon suture as described in the "animal preparation" section) to examine the MTrS in different directions. When the search needle approached an active locus of the MTrS, a continuous distant electrical activity could be seen and heard. An EPN amplitude higher than 10 μV was counted as positive EPN (Figure 4). If there was no electrical activity, the search needle was advanced to a small distance (approximately1 mm) to find another active locus. After five movements in one direction (one track), the search needle was withdrawn to its starting point and then redirected to penetrate unexplored muscle tissue in a second track and advanced five more times, as described above. Five tracks were explored to complete exploration of a cone-shaped space. This allowed for the exploration of 25 different points in the region of each MTrS. Thus, the EPN prevalence was calculated as the number of positive EPNs divided by 25.

During the procedure of EPN measurement, the search needle was advanced and rotated very slowly to avoid it from "grabbing" the tissue and releasing it suddenly, which would cause a large jump. Large movements were avoided because they could induce local twitch responses. At each follow-up measurement, the skin and muscle conditions were observed. If there was

hematoma, bleeding or lost of nylon suture, it was recorded.

Data analysis

One-way ANOVA was used to compare values among all groups. The percentage changes in EPN after 1st and 5th treatment were normalized using the following formulae:

$Change_1$ (%) = (post-1st treatment −pretreatment)/(pretreatment)×100%

$Change_2$ (%) = (post-5th treatment − pretreatment)/(pretreatment)×100%

$Change_3$ (%) = (post-5th treatment − post-1st treatment)/(post-1st treatment)×100%

Repeated-measured ANOVA was used to investigate the immediate (post-1st treatment) and cumulative (post-5th treatment) effects in each group by comparing pre-treatment, post-1st treatment, and post-5th treatment values. A p value of <0.05 was considered to be statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences Version 18.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

We initially collected 37 New Zealand rabbits (74 legs) but excluded 25 legs because their EPN prevalence was <24%. In addition, from the 2nd to the 5th sessions, we excluded two rabbits (four legs) in the 830 nm group because of bilateral intramuscular hematomas in the tested muscles and two rabbits (four legs) in the 980 nm group because the markers of the MTrS were lost in one rabbit and one rabbit died before the 5th day. Thus, we calculated outcome measurements based on the remaining 41 legs (Figure 1). The three groups had similar body weights and pre-treatment EPN prevalence (p>0.05, Table 2).

Comparing values before treatment and post-1st treatment indicated significantly decreased EPN prevalence in each group (p<0.05 for all) but did not indicate any significant differences among these groups (Table 3). This suggested that these two settings (830nm, 980nm) of LLLT did not immediately affect the irritability of MTrSs.

After five treatments, there was also significantly decreased EPN prevalence in each group relative to the pretreatment values (p<0.05 for all) but no significant differences were seen among the groups (Table 3). There were also no significant differences between the values at the post-1st and post-5th treatments. The results indicated that the applied LLLT did not have a cumulative effect on the irritability of MTrSs.

Table 1. The parameters of low level laser therapy in the presented study

Parameters	Setting in the presented study
Number of laser diodes in the probe	6 laser diodes
Wavelength (nm)	830 nm in 830 group, 980 nm in 980 group
Frequency (Hz)	continuous
Spot size of laser (cm^2) – each diode	0.01 cm^2 in 830 group; 0.02 cm^2 in 980 group
Area of probe (cm^2) – probe	12.25 cm^2
Power output (mW) – each diode	30 mW in 830 group; 50mW in 980 group
Power output (mW) – probe	180 mW in 830 group; 300mW in 980 group
Power density (mW/cm^2) – each diode	18000 mW/cm^2 in 830 group; 9000 mW/cm^2 in 980 group
Power density (mW/cm^2) – probe	14.69 mW/cm^2 in 830 group; 24.49 mW/cm^2 in 980 group
Irradiation time(sec)	34sec in 830 group; 20 sec in 980 group; 20 sec in placebo group
Energy in each session (J)	6 J in both 830 group and 980 group; 0 J in placebo group
Application mode	0.5cm perpendicular to the skin

Table 2. Demographic data at baseline

Group	Placebo	830 nm	980 nm	p value[1]
Number of legs	14	12	15	
Body weight (kg)	3.42 ±0.32	3.45±0.49	3.50±0.19	>0.005
Prevalence of EPN(%)	28.86±7.22	26.33±2.67	29.07±5.34	>0.005

NOTE. Values are mean ± standard deviation or p value.

[1]One-way ANOVA with Bonferroni for post-Hoc analysis to compare the values among different groups.

Abbreviations:

EPN, endplate noise

Table 3. Comparison of prevalence of endplate noise at 3 time points: before treatment, after the 1st and the 5th low level laser therapy.

Group	N		Immediate effect		Cumulative effect			p value[2]
		Pre (%)	Post-1st (%)	$Change_1$ (%)	Post-5th (%)	$Change_2$(%)	$Change_3$(%)	
Placebo	14	28.86±7.22$^{AB, AC}$	16.57±12.24^{AB,BC}	-37.02±53.75	16.00±7.36^{AC,BC}	-41.49±31.40	46.52±104.19	AB=0.013* AC=0.001** BC=0.872
830 nm	12	26.33±2.67$^{AB, AC}$	13.67±9.57$^{AB, BC}$	-46.97±37.30	16.67 ±10.07^{AC,BC}	-36.46 ±37.70	121.41±290.09	AB=0.002** AC=0.007** BC=0.476
980 nm	15	29.07±5.34$^{AB, AC}$	14.67±8.51^{AB,BC}	-48.24±29.77	14.93±7.63^{AC,BC}	-46.38±32.00	43.95±149.96	AB<0.001** AC<0.001** BC=0.932
p value[1]		>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	

NOTE. Values are mean ± standard deviation, or p value.

*p< 0.05, **p< 0.01

[1]One-way ANOVA with Bonferroni for post-Hoc analysis to compare the values among different groups.

[2]Repeated measured ANOVA was used to compare the values within each group.

Abbreviations:

N, number of leg; EPN, endplate noise; Pre, EPN prevalence before treatment, %;

Post-1st, EPN prevalence immediate after the 1st treatment, %; Post-5th, EPN prevalence after the 5th treatment, %;

$Change_1$ (%), (post-1st treatment − pretreatment)/(pretreatment)×100%

$Change_2$ (%), (post-5th treatment − pretreatment)/(pretreatment)×100%

$Change_3$ (%), (post-5th treatment − post-1st treatment)/(post-1st treatment)×100%

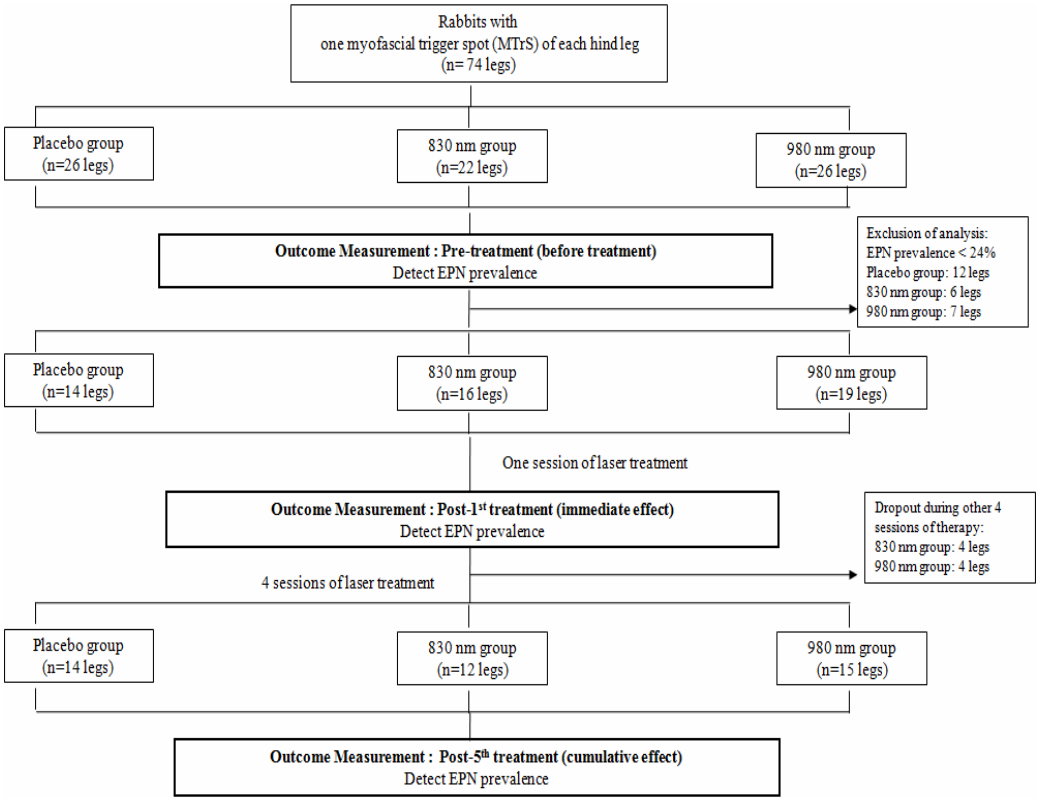

Figure 1. Flow chart of study design.

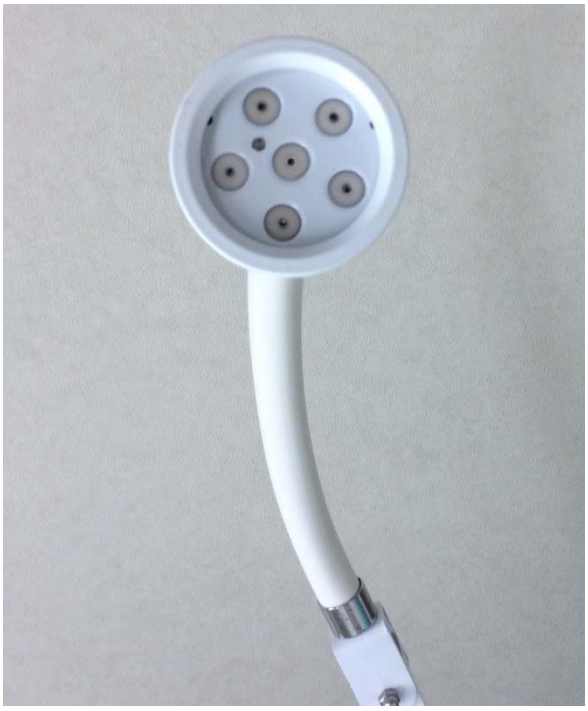

Figure 2. The probe of cluster type laser.

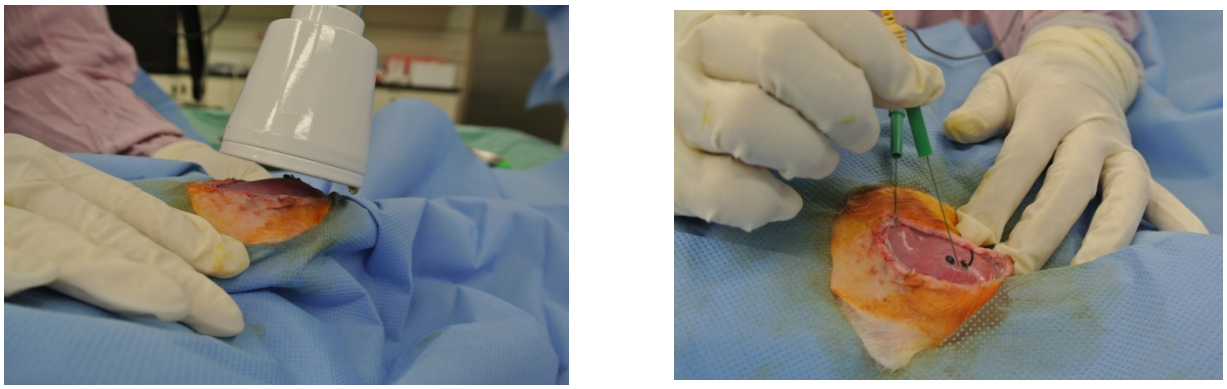

Figure 3. The application of laser therapy (left) and the assessment of endplate noise (right).

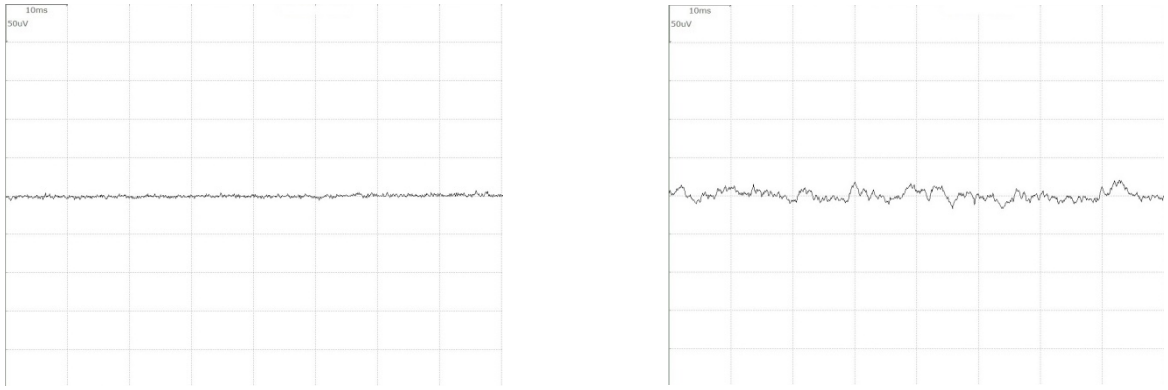

Figure 4. Baseline electromyographic figure (left) and typical electrical activity of endplate noise (EPN) (right).

DISCUSSION

In this study, we found a significantly reduced prevalence of EPN (irritability) in all groups after both one and five sessions of LLLT. However, there was no statistically significant difference between the experimental groups and the control group at any follow-up time. These results indicated that the cluster probe LLLT used in this study did not have a significantly better inhibitory effect on the rabbits' MTrSs than placebo therapy.

Possible reasons for the negative result

The effects of different wavelengths of LLLT are variable. In Lin et al (2000), a continuous wavelength of 632nm LLLT was reported that the pain threshold was improved in the target muscle.[30] A systemic review by Chow and Barnsley[31] reported that several wavelengths of LLLT (780 nm, 810–830 nm, 904 nm, and 1064 nm) significantly relieved neck pain. However, they also noted significant heterogeneity in outcome measures and in treatment characteristics based on the reported results as well as doses and laser parameters achieved in the study. Hashmi et al[32] reviewed LLLT and concluded that continuous wavelength LLLT had some effects that were different from pulsed LLLT. However, a Cochrane review concluded that there are insufficient data to draw firm conclusions about the clinical efficacy of LLLT because of the heterogeneity of study populations and interventions as well as different groups used for comparisons.[33] In our previous studies,[27, 28] we found that the effect of LLLT on MTrSs depended on the energy (dose) of the laser. In these two studies, a single spot (spot size: 0.2 cm^2) diode laser with a continuous wavelength of 660 nm was used. In the 2008 study, we reported the immediate and cumulative effects of LLLT on EPN prevalence in MTrSs in rabbits after a single dose (energy of 1.8 J/session) and after six sessions (total energy of 10.8 J).[27] In the study by Chen et al,[28] the same laser was investigated using different energies in

two groups of rabbits (energy of 5.4 J/session in a low-dose group and of 14.4 J/session in a high-dose group). We found a dose-dependent effect in the low-dose group but observed a ceiling effect in the high-dose group. In the current study, most of the settings and procedures were similar to those in our previous studies.[27, 28] However, we used a cluster probe laser rather than a spot laser in the present study. The energy of the laser in the present study was 6J/session and 30J over five sessions in the two experimental groups. This dose was within the effective range used in our previous studies (1.8–14.4 J/session).[27, 28] This study failed to demonstrate a therapeutic effect of laser treatment on MTrS irritability. One of the causes is related to the different type of laser probe. With a single spot laser probe, the laser light came from one laser diode and the laser light was easily placed on the taut band directly. Thus, the energy could be absorbed in the taut band and cause a serial cellular response. In contrast, there were six laser diode spots in one cluster probe in the present study. The spot size of each laser diode was 0.01–0.02 cm^2, and the total area covered by each probe was 12.25 cm^2. There were six laser lights applied to the tested muscle. Only one laser light was applied directly to the taut band region, and the other five laser lights were applied to the non-taut band region. The laser energy on the non-taut band region may not penetrate into the taut band region. Therefore, there was no therapeutic effect on the irritability of MTrSs in the present study.

In current study, the laser lights from these 6 laser diodes were parallel to each other. In this design, the main benefit was that it can treat the lesion with multiple target sites simultaneously. If there were multiple lesions in one muscle, it could shorten the treatment time. In contrast, this benefit did not appear if the lesion was small or the target site was single, such as in our study. Therefore, the design of cluster probe LLLT may need to be modified in the future. We hoped that the output angle of laser light from each laser diode can be adjusted by the operator. Thus, the area of laser light can be focused on different size of the lesion and the indications for using cluster LLLT will be more in the future.

Other possible causes of decreased EPN prevalence in all 3 groups:

In this study, we found that the EPN prevalences of the control group and two experimental groups were decreased after the 1st and the 5th treatment. In addition to the different probe LLLT, there might be other coexisted causes leading to this change. These coexisted possible causes might be related to the muscle relaxation under anesthesia, the effect of analgesics and immobility after surgery. As the measurement of EPN prevalence should be in alive animals and in the same MTrS in this study, these procedures were performed after adequate anesthesia and analgesics. There was also a marked taut band, which encircled with 3-0 nylon suture. During the daily observation of the rabbits, we noticed that the activity of them became less. Therefore, these medications and post-operative immobility lead to other possible causes of decreasing EPN prevalence after the 1st and the 5th treatment. Because of these reasons, this study should include control group (no LLLT).The difference between control and experimental groups should be controlled to one factor only (received LLLT or not) in order to investigate the effect of LLLT.

The World Association of Laser Therapy (WALT) recommendation

Could we follow the recommendation from the WALT in the present study? Based on the heterogeneity of laser studies, WALT suggested that scientific articles should follow a "Consensus agreement on the design and conduct of clinical studies with low level laser therapy and light therapy for musculoskeletal pain and disorders."[34] Updates on optimal treatment was available at the WALT website (www.walt.nu). The recent updated agreement stated that authors should document the following parameters of LLLT: wavelength (unit: nm), average output (unit: mW), treatment time (unit: seconds), reported energy dose delivered (unit: J/cm^2), spot size (unit: cm^2), power density (unit: mW/cm^2), and accumulated energy (unit: J). For single diode spot laser probes, all these parameters can be documented easily and precisely. However, the calculations for power density and energy dose involve dividing the power output and energy by spot size. As described above, there were six laser diode spots in one cluster probe. The spot size was substantially smaller than the probe output area (0.01–0.02 cm^2 vs.12.25 cm^2). The energy density of each diode spot would be 612 J/cm^2 in the 830nm group

(energy density (J/cm^2) = (power output (mW) × irradiation time (sec))/(spot size (cm^2) × 1000) = 180 × 34/(0.01 ×1000)). If the energy density of the total laser output of the probe is calculated, it would be 0.4996 J/cm^2 in the 830nm group (energy density = (180×34)/(12.25 × 1000)). There was considerable difference between the two above mentioned results (612 J/cm^2 vs. 0.4996 J/cm^2). Thus, we preferred to report the energy (unit: J) and designed the study using the same energy as with our previous studies.[27, 28] For the same reason, the WALT-recommended dose of LLLT expressed as energy density might not be suitable for cluster probe LLLT.[34] As there is no standard dose for this method, future studies to evaluate therapeutic parameters for cluster probe LLLT are warranted.

Limitations

This animal study firstly used cluster probe LLLT in a myofascial pain model. No reference for determining the effective energy dose was the major limitation. Following the rules in our previous animal studies by using a single spot diode laser was not available. The optimal setting for a cluster probe LLLT is also unknown. There was no therapeutic effect of cluster probe LLLT in this study. However, the possibility of therapeutic effects with higher doses or of using other settings of the same device cannot be excluded. Further research for studying the effects of LLLT with other parameters, such as higher energy, different combinations of wavelengths, or different frequencies of energy output, would be used.

CONCLUSION

In this study, the cluster probe LLLT with continuous wavelength at 830 nm and 980 nm (energy of 6J/session or total energy of 30J over five sessions) did not have a significantly better inhibitory effect on the rabbits' MTrSs than placebo therapy.

ACKNOWLEDGEMENTS

The authors acknowledge the funding by Chang Gung Memorial Hospital, Chiayi, Taiwan (grant number: CMRPG6B0441 and CMRPG6B0442). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors declare that there are no conflicts of interests regarding the publication of this paper.

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探討以叢集式低能量雷射對於大白兔肌筋膜激痛點之效應：個案控制型研究

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研究目的：本研究探討以波長 830nm 或 980nm、持續性輸出、聚集式低能量雷射對大白兔骨骼肌肌筋膜激痛點之效應。研究方法：以 37 隻紐西蘭大白兔分爲 3 組，分別給予波長 830nm 的雷射治療(6 焦耳/腳/天)、波長 980nm 的雷射治療(6 焦耳/腳/天)、對照組(0 焦耳/腳/天)的治療共 5 天。在治療前、第一次及第五次治療後以肌電圖針測量每一肌筋膜激痛點的終板電板(endplate noise, EPN)的盛行率。研究結果：各組的終板電板盛行率隨時間而減少。但於第一次治療後或第五次治療後三組間並無差異。結論：三組的肌筋膜激痛點的盛行率於第一次治療後及第五次治療後都出現下降情形，但三組間並沒有統計上的差異。此結果表示，以此設定模式的叢集式低能量雷射對於大白兔骨骼肌肌筋膜激痛點抑制效應(inhibitory effect)，兩組實驗組均無法優於對照組。 (台灣復健醫誌 2015；43(4)：251 - 262)

關鍵詞：聚集式探頭(cluster probe)，低能量雷射治療(low level laser therapy)，肌筋膜疼痛(myofascial pain)，兔子(rabbit)，波長(wavelength)

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