PLASMA ACTH AND β-ENDORPHIN LEVELS IN RESPONSE TO LOW LEVEL LASER THERAPY (LLLT) FOR MYOFASCIAL TRIGGER POINTS

E. Liisa Laakso1, Tess Cramond2, Carolyn Richardson3 and John P. Galligan4

1: Physiotherapy Department, Royal Brisbane Hospital, 2: Pain Clinic, Royal Brisbane Hospital, 3: Physiotherapy Department, University of Queensland, and 4: Chemical Pathology Department, Royal Brisbane Hospital, Queensland, Australia.

The mechanism by which laser phototherapy (Low Level Laser Therapy - LLLT) induces analgesia in the treatment of chronic pain is not understood. To investigate a possible role for opioids in this treatment, a double-blind, placebo-controlled study was designed to compare the effect of two dosages (1 J/cm² and 5 J/cm²) of an infrared (IR) laser (820 nm), a visible red laser (670 nm) and a near-monochromatic light emitting device (660 nm, 30 nm bandwidth) on trigger points. Fifty-six consenting subjects with chronic pain conditions exhibiting myofascial trigger points in the neck and upper trunk region underwent six experimental sessions over a two week period. Blood samples were withdrawn before and after treatment on three of six appointments. Plasma was assayed for β-endorphin (radioimmunoassay, RIA) and adrenocorticotropic hormone (ACTH - two-site immunoradiometric assay, IRMA) to assess opioid response. ACTH was shown to have a cumulative response to treatment with a significant response to a 1 J/cm² infrared laser (p < 0.001) and a 5 J/cm² red laser (p < 0.05). β-endorphin was noted to be significantly elevated between days one and four (p < 0.05) in subjects who received IR (5 J/cm²) laser therapy. Results indicated that the analgesic response to phototherapy may be mediated through hormonal/opioid mechanisms, and that responses to LLLT are dose and wavelength dependent. A mechanism is proposed by which peripheral stimulation using LLLT may elicit activity in the central pathways.

Key Words: Analgesia, chronic pain, cytokines, Low Level Laser Therapy, opioid mechanism; phototherapy

Introduction

For many years, Low Level Laser Therapy (LLLT) has been used clinically in the management of chronic pain conditions. Research has demonstrated variable responses, which may have been due to the variety of doses, wavelengths and study designs utilized. The variability in the parameters used has made comparison of laser studies a difficult task.

Some authors document the use of LLLT in the successful treatment of myofascial trigger points and related disorders, but with no adequate explanation of how relief may be obtained. Walker,7 using the helium neon laser, reported an increase in urinary 5-hydroxyindoleacetic acid (5-HIAA) in subjects who gained symptomatic relief of various chronic pain disorders. She suggested that the central descending inhibitory pathways (and the endogenous opioids) may be implicated in the mechanism of action of LLLT. Even though Walker’s findings have not been replicated,8 many still cite her work as support that not only does LLLT have a systemic effect (supported by Mester et al),9 but also that the opioid system is involved.

It is believed that the effects of LLLT are dose, power and wavelength dependent.10 Some suggest that a therapeutic window exists between energy densities of 0.5 J/cm² and 4 J/cm²11-13 and that doses above this window may result in inhibition of cell processes. Others maintain that it is not necessary to use true laser (with the unique properties of collimation, coherence and monochromaticity) to achieve results, but that near-monochromatic light is sufficient.14,15

Despite the clinical evidence, research has both failed to confirm these ideas unequivocally, or to propose an adequate mechanism by which LLLT ameliorates pain. This therefore was the first study since Walker7 to investigate a possible link in human subjects, between LLLT and a central opioid mechanism, perhaps through a link between the stress system and the immune system. ACTH and peripheral β-endorphin were chosen in order to reflect possible central responses to peripheral stimulation with LLLT. The study utilized two different doses (1 J/cm² and 5 J/cm²) and
two different wavelengths of laser (670 nm and 820 nm), as well as near-monochromatic (non-laser) red light (660 nm), to assess the effect of phototherapy on adrenocorticotropic hormone (ACTH) and \( \beta \)-endorphin of patients suffering from the chronic pain associated with myofascial trigger points.

Materials and Methods

Subjects

Fifty-six subjects suffering chronic pain conditions characterized by myofascial trigger points of the neck, shoulders and upper thoracic regions were recruited by community advertisements and contacts with local doctors and physiotherapists.

Subjects were included only if they had a recent medical diagnosis which included fibrositis, myofascial pain syndrome, myositis and overuse syndrome. If subjects had other underlying pathologies they were excluded. Subjects were aged over eighteen years (mean = 41.95 years) and included both females (n=40) and males (n=16). An additional requirement was that subjects must have had pain for at least six months (mean = 7.5 years).

Potential subjects were excluded if they had a history of narcotic use, malignancy, cranial surgery/trauma, coagulopathy or were currently engaged in compensation/litigation issues, or taking photosensitizing agents or other concurrent treatment (including physiotherapy). Subjects were not excluded if they used occasional simple analgesia but were disqualified if they were taking tricyclic anti-depressants, non-steroidal anti-inflammatory agents or other drugs.

As the ability of certain wavelengths of laser to penetrate pigmented tissue is questionable, those subjects who were well-tanned or tattooed in the area of pain were also excluded. Participation in the study was voluntary, and each subject was fully informed of the procedure of the study (including the possibility of side effects) prior to giving written consent.

Subjects were randomly allocated to one of eight treatment groups (Table 1). Each subject was identified by a code number signifying the treatment probe to be used and the treatment time to be applied. The code was broken only at the completion of the study.

Procedure

All testing and treatment was performed at the Royal Brisbane Hospital Physiotherapy Department, and the Primary Care Section of the Accident and Emergency Department. Room temperature remained constant at 23°C. To avoid stress, subjects were provided with nearby parking. Subjects underwent testing and treatment according to the schedule outlined in Table 2. There was no treatment intervention on the first day of the study (Friday). Treatment was carried out three times during the following week (Monday, Wednesday and Friday) and twice in week two of the study (Monday and Wednesday). To control for circadian variation of plasma ACTH and \( \beta \)-endorphin, treatment sessions occurred at times which were as close as possible to the first appointment time. The protocol of the study was approved by the Royal Brisbane Hospital Research Ethics Committee and University of Queensland Ethics Committee.

Equipment

The laser used in the study was an Intellect 800 (Class IIIB laser product, Chattanooga Australia Pty. Ltd.), and for the purposes of this study it was equipped with four probes: 1 gallium-aluminium-arsenide (GaAlAs) 820 nm (infrared - IR) 25 mW semiconductor laser diode (spot size: 0.028 cm², beam divergence of 6°) (Groups 3a and 3b); 2 non-operating probe for placebo treatment (Groups 4a and 4b); 3 670 nm (red) 10 mW semiconductor laser diode (spot size: 0.036 cm²) (Groups 2a and 2b); and 4 660 nm 9.5 mW monochromatic (red) light emitting diode (LED, spot size: 0.031 cm²) (Groups 1a and 1b).

Because it was not possible to obtain identical wavelengths for the visible red laser and near-monochromatic red light probes, wavelengths as close as possible were chosen.

The specific effects of various pulsing frequencies...
of LLLT have not yet been established. Other studies\(^{(16,17)}\) have used a pulse frequency of 5000 Hz. To ensure that power output remained constant between treatments, the same pulse frequency was chosen. The unit and probes were tested before and after the study using a Jobin-Yvon monochromator and Newport power meter. No deterioration in power output or light quality was detected. To preserve the double-blind format of the study, treatment probes were identical in appearance, and the disabled placebo probe caused the laser unit to respond in the same manner as would an active probe (i.e., illumination of the front panel and emission of an audio signal).

### Treatment

Each subject was supplied with a topical anaesthetic cream (lignocaine and prilocaine 25 mg/g) applied one hour prior to venipuncture to help reduce unwanted discomfort or stress. Blood sampling was performed by trained staff (blind to the study protocol) by venipuncture of the antecubital vein. Subjects were asked to undress sufficiently to expose the area of treatment. For all sessions, subjects were comfortably but uniformly seated in a chair with armrests, avoiding unnecessary stretch on the neck and shoulder musculature. At the commencement of each treatment session, subjects were asked to isolate the three most painful trigger points in the area in question. Trigger points were defined and located according to the protocol outlined by Snyder-Mackler et al.\(^{(4)}\) Subjects were asked to locate painful areas and the author then palpated to locate hypersensitive trigger points which referred pain. Each of the trigger points was circled using a soft marking pen. Treatment was applied by a physiotherapist blind to the study protocol. Both subject and physiotherapist wore protective glasses during treatment. Once positioned, subjects were asked to refrain from movement. Treatment was applied with the laser probe aperture lightly resting on the skin surface within the circle marking the trigger point. The same dose was applied to each trigger point.

### Assay of Blood Samples

It is acknowledged that levels of β-endorphin from plasma collected peripherally do not necessarily reflect central release patterns of this substance. Due to ethical and logistical considerations, collection of cerebrospinal fluid (CSF) samples was not feasible therefore only measurement of peripheral β-endorphin levels was performed in this study. Assay of plasma β-endorphin is difficult. To prevent a potential lack of usable results, ACTH was measured also. Both β-endorphin and ACTH share the common precursor proopiomelanocortin (POMC)\(^{(18)}\) and so ACTH was considered to be an appropriate indicator of opioid activity. Diurnal variations in plasma levels of β-endorphin and ACTH were controlled by treating patients and collecting blood samples as near as possible (i.e., 30 minutes either side) to the initial treatment time.

Radioimmunoassay (RIA) for β-endorphin was carried out using an antibody and following the assay technique in use at the Baker Medical Research Institute (Melbourne), and which has been described previously.\(^{(19)}\) The β-endorphin RIA was performed under disequilibrium conditions with a specific β-endorphin antiserum (R56, final dilution 1:54000). Human and ovine β-endorphin were equipotent in the RIA. Cross-reactivity to β-LPH was 50% on a molar basis. The cross-reactivities of other peptides tested were all < 0.1% (i.e., met-enkephalin, leu-enkephalin, ACTH 1-39, CLIP, α-MSH, α-endorphin and γ-endorphin). The sensitivity of the RIA was 2 pg/tube.

Two-site immunoradiometric assay (IRMA) for ACTH was carried out in the Chemical Pathology Department of the Royal Brisbane Hospital, using a standard commercially available kit (Nichols Institute Diagnostics ACTH 65T Kit, Cat. No: 40-2194). There was no cross-reactivity with ACTH 1-24, ACTH 11-24, ACTH 18-39, ACTH 1-10, α-MSH, β-MSH, β-LSH or β-endorphin. The sensitivity of the IRMA was 2 ng/l.

### Preparation of Blood Samples

Eighteen millilitres of blood was collected from each subject at each blood test. Ten millilitres of blood was collected into a plastic, heparinized tube containing 500 μl of β-endorphin inhibitor mix (200 ml inhibitor mix = 40 ml Sigma Aprotinin (Cat No A6279), 5g N-ethyl maleimide (NEM), 3.72g Na₂EDTA.2H₂O and 160 ml 0.9% saline). The remaining 8 ml was divided into EDTA tubes for ACTH assay. All tubes were placed on ice, and centrifuged within 10 minutes (in a DAMON/IEC centrifuge at 3000 rpm (4°C) for 12 minutes). Resultant plasma was stored in labelled 5 ml plastic tubes, and frozen at -70°C until assay.

### Data Analysis

Seven subjects did not complete the study. A computer program (Statistical Analysis System - SAS) was used to analyze the results. The main statistical examinations were for analysis of variance (ANOVA, using the General Linear Models Procedure and post hoc Duncan’s Multiple Range Test), and Pearson Product Moment for correlation analyses. The variables and interactions tested were Wavelength, Dose and Day. Independent Student’s t-tests were used to assess levels of significance between statistically significant interactions.

### Results

As expected and due to their common origin, plasma
levels of ir-ACTH and ir-β-endorphin were highly correlated for all wavelength and dose groupings, as shown in Table 3. Plasma levels of ACTH and β-endorphin were within the normal range for adult humans. The correlation coefficient for near-monochromatic red light was half that observed for other treatment groups. Table 4 presents the degrees of freedom, error, F values and the level of significance for the analyses of variance between groups. The main feature to note is the high level of significance for differences in plasma biochemistry between groups, based on wavelength and dose regressions.

**ACTH**

Analysis of the pattern of release of ACTH in each experimental group for each blood testing day during the trial (Figures 1 and 2) demonstrated the cumulative increase of pre-treatment ACTH levels between days one and six, in the groups which received IR laser at 1 J/cm² (p < 0.001, t-test) and red laser at 5 J/cm² (p < 0.05, t-test). Infrared laser at 5 J/cm² demonstrated a similar trend (p < 0.05, t-test). ACTH levels for the (5 J/cm²) red laser group commenced higher than, and remained higher than placebo ACTH levels throughout the duration of the study, whereas ACTH levels for (1 J/cm²) IR laser commenced lower, but finished significantly higher than placebo levels (Figure 2). In all groups except (1 J/cm²) placebo on Day Six, and (5 J/cm²) near-mono-

---

**Table 3:** Correlation coefficients (Pearson Product Moment) for ACTH and β-endorphin for each wavelength (λ) and dose grouping

<table>
<thead>
<tr>
<th>λ/Dose Grouping</th>
<th>β-Endorphin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall ACTH</td>
<td>0.7†</td>
</tr>
<tr>
<td>Near monochromatic red light ACTH</td>
<td>0.34‡</td>
</tr>
<tr>
<td>Red laser ACTH</td>
<td>0.69†</td>
</tr>
<tr>
<td>Infrared laser ACTH</td>
<td>0.7†</td>
</tr>
<tr>
<td>Placebo ACTH</td>
<td>0.85‡</td>
</tr>
<tr>
<td>High dose treatment ACTH</td>
<td>0.69‡</td>
</tr>
<tr>
<td>Low dose treatment ACTH</td>
<td>0.7†</td>
</tr>
</tbody>
</table>

†, p<0.0001; ‡, p<0.01

**Table 4:** Summary of ANOVA statistics for ACTH and β-endorphin

<table>
<thead>
<tr>
<th>Source/Interaction</th>
<th>DF</th>
<th>F Value ACTH</th>
<th>F Value β-endorphin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>3</td>
<td>2.68†</td>
<td>3.37†</td>
</tr>
<tr>
<td>Dose</td>
<td>1</td>
<td>3</td>
<td>0.48</td>
</tr>
<tr>
<td>Subject (λ &amp; dose)</td>
<td>48</td>
<td>6.58‡</td>
<td>5.52‡</td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>2.12</td>
<td>0.04</td>
</tr>
<tr>
<td>Day</td>
<td>2</td>
<td>0.43</td>
<td>0.02</td>
</tr>
<tr>
<td>λ x Dose</td>
<td>3</td>
<td>8.44††</td>
<td>7.19††</td>
</tr>
<tr>
<td>Dose x Time</td>
<td>1</td>
<td>0.92</td>
<td>0.68</td>
</tr>
<tr>
<td>Dose x Day</td>
<td>2</td>
<td>1.17</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Error: 248

†, p<0.05; ‡, p<0.01; ††, p<0.0001

---

**Fig 1:** Low dose experimental groups: analysis of measurements of ACTH (day, dose and wavelength interaction).
chromatic red light on day four, immediate post-treatment measures of ACTH were not significantly lower than pretreatment levels.

β-endorphin
Figures 3 and 4 demonstrate the patterns of release for β-endorphin. There was a significant cumulative increase (p< 0.05, t-test) in pre-treatment β-endorphin levels for the group which received (5 J/cm²) IR laser. The trend was similar to that noted for the corresponding ACTH measurements (Figure 2). All other changes in β-endorphin levels were not statistically significant.

Discussion
The findings of this study suggest that earlier theories may have been correct and that it may be possible to activate a central release of ACTH and β-endorphin via a peripheral stimulus, namely LLLT. However, the link between the peripheral stimulus and the central re-
Response is not completely understood and it must be acknowledged that levels of peripherally circulating β-endorphin and ACTH may not accurately reflect central responses.

Proposed Mechanism of Pain Attenuation

Previous studies have shown that mast cell degranulation occurs using LLLT(20,21) and that macrophages can be stimulated to release inflammatory mediators(22,23) Interleukin-1 (IL-1) one of the lymphokynes, is one such mediator. Interleukin-1 has been shown to be capable of causing a central release of corticotropin-releasing hormone (CRH) from the hypothalamus(24) and secretion of ACTH from the pituitary.(25) It is known that activation of the hypothalamic-pituitary-adrenal (HPA) axis results in stimulation of the sympathetic outflow, which in turn results in immunosuppression.(26) Central activation of CRH also activates proopiomelanocortin (POMC) to counter-regulate CRH and sympathetic system activity, and to induce opioid receptor-mediated, stress-related analgesia.(27) Other studies have confirmed that a systemic or humoral effect can be stimulated with peripheral LLLT. Goldman et al.(28) treated patients with rheumatoid arthritis and found that the level of circulating immune complexes (as measured by platelet aggregation) was affected by laser therapy. The same authors cite others who found that immunoglobulins were influenced by LLLT of wounds. These examples suggest that there is a link between local peripheral stimulation using LLLT and activation of the central immune system.

Although specific changes in β-endorphin levels during this study were not large, except for near-monochromatic red light, there was a very high positive correlation between ACTH and β-endorphin levels for all wavelengths and doses. This finding suggests that a common precursor to both ACTH and β-endorphin was stimulated by the treatments administered in this study.

The findings of this study reinforce the suggestion that light at certain wavelengths and doses provides a means of stimulating cellular processes.(22,29) The findings also indicate that in order to stimulate central release of ACTH, the window of therapeutic doses should be inclusive of 5 J/cm² energy density. Relatively high energy densities (i.e., 5 J/cm²) may have resulted in mast cell degranulation, or macrophages may have been stimulated to release inflammatory cytokines, in order to stimulate immunosuppression through activation of the HPA axis. It is possible that the higher power density achieved by using a 25 mW IR diode laser may have been the critical parameter of effect on the final results. This factor was not able to be controlled due to difficulties in obtaining suitable equipment.

Of interest was the cumulative increase of pre-treatment ACTH levels in the groups which received (1 J/cm²) IR laser and (5 J/cm²) red laser, and cumulative increase in pre-treatment β-endorphin levels for the group which received (5 J/cm²) IR laser. No other groups exhibited these changes. The more significant increase in ACTH levels noted in the group which received (1 J/cm²) IR laser would suggest that the response to (1 J/cm²) IR laser was more significant than that of (5 J/cm²) red laser.

A cumulative response to LLLT has been proposed by some authors(30) and these results would confirm their notion. The fact that such a cumulative response was noted only in laser-treated groups suggests that true laser light was necessary to produce such changes, i.e., that near-monochromatic red light was not suf-fi-
cient. This remains to be more specifically tested.

If (1 J/cm² and 5 J/cm²) IR laser, and (5 J/cm²) red laser energies were able to stimulate the release of cytokines from macrophages or if mast cell degranulation resulted in an increase in local inflammatory mediators, then a link between the periphery and the resultant central release of ACTH and β-endorphin could be proposed. Repeated treatment with (1 J/cm² and 5 J/cm²) IR laser and (5 J/cm²) red laser, over a number of days, may have served to reinforce this effect thus resulting in a cumulative increase of pretreatment ACTH and β-endorphin levels.

It may be argued that peripheral circulatory products would be unable to cross the blood-brain barrier. The circumventricular organs (CVO) lining the cerebral ventricles are in a position to influence the centres controlling not only thermoregulation and sleep but also secretion of hormones. It has been proposed that the “capillary leak syndrome” may provide a direct pathway for the passage of cytokines to the central nervous system by dramatic shift of fluid from the capillary bed. If this was possible, the final link between the periphery and the higher centres would be revealed.

ACTH levels (and to a lesser extent, β-endorphin levels) were lower after treatment in almost all groups. If subjects had been stressed by the study protocol, ACTH levels would have increased. Stress can therefore be discounted as a variable which may have influenced the results of this study. ACTH levels decreased significantly after treatment in only two groups (1 J/cm² IR laser and 5 J/cm² red laser, both on day six). This post-treatment response may have been due to a perception by subjects that they were receiving a positive benefit from treatment (regardless of whether they received placebo or active treatment), or perhaps less likely due to circadian variation. The groups which did not demonstrate post-treatment ACTH reductions showed no variations in post-treatment measures (Figures 1-4). Pre- and post-treatment β-endorphin levels did not vary significantly in any group.

An alternative mechanism by which the previously described pre-treatment ACTH and β-endorphin levels were increased may have been through stimulation of local CRH. Chrousos(26) has detected immunoreactive CRH intracellularly in inflamed tissue and suggests that local production of CRH occurs in sufficiently high concentrations “... to produce biologic responses including POMC-derived peptide secretion and regulation of immune function”. Chrousos further hypothesized that locally produced CRH can stimulate β-endorphin for antinociception, “... by exerting its own direct antinociceptive effects in the inflammatory site, or by participating in the transmission of pain sig-
dorphin and ACTH, it is suggested that the therapeutic window of doses for LLLT treatment of trigger points could be extended to include 5 J/cm². This would need to be validated by conducting adjunctive studies on subjective pain responses. It is acknowledged that power density may have resulted in the fact that neither low dose nor high dose near-monochromatic red light (660 nm) was found to be capable of eliciting significant changes in blood biochemistry. The suggestion that the laser is a necessary requirement for phototherapy of trigger points remains to be confirmed.

Acknowledgements

“This research was made possible by a scholarship from the Sir Robert Menzies Memorial Foundation, and grants from the Dorothy Hopkins Committee, Queensland Branch of the Australian Physiotherapy Association, the Royal Brisbane Hospital (RBH) Research Foundation, and Queensland Health.”

The authors thank the Sir Robert Menzies Memorial Foundation for their support; Chattanooga Australia Pty., Ltd., for assisting in the procurement for purchase of suitable equipment; Ms Anne Kelly, Ms Maree Raymer and staff of the Physiotherapy Department, RBH, for their invaluable assistance and cooperation; Mrs Meryl Fullerton and Prof. John Funder at the Baker Medical Research Institute, and staff of the RBH Chemical Pathology Department, for their valuable time and assistance in blood assays; and medical, nursing and other staff of the RBH and University of Queensland who made the study possible.

References


