MORPHOLOGICAL DEMONSTRATION OF LOW REACTIVE LASER THERAPEUTIC PAIN ATTENUATION EFFECT OF THE GALLIUM ALUMINIUM ARSENIDE DIODE LASER

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It is possible to increase the volume of mitochondria in certain types of nerve cells with free nerve endings which are sensitive to pain-producing substances. In order to achieve this increase, the substance must be high-density, and be in contact with the cell for a prolonged period. There is also a positive correlation between the increase in the frequency of nerve impulses and the increase in the mitochondrial volume. In this study, bradykinin was used as the pain-producing substance. Three groups of animals were used. A high concentration of bradykinin was injected subcutaneously into the maxillary trigeminal dermatome of male Wistar rats over a 4-day period. One group received bradykinin only, one group received bradykinin followed by irradiation with a diode laser, and a third group received only physiologic saline as a control. After the twelfth day, the animals were killed. The mitochondrial density of the trigeminal ganglion cells and the synaptic glomeruli of the spinal trigeminal nucleus was checked. The volume was significantly high in group 1, but in group 2 was almost the same as the normal level of control group 3. A significant statistical difference was demonstrated. From these findings it is possible to conclude that the diode laser can reduce the pain-producing action of bradykinin in peripheral nerve tissue.

KEY WORDS: LLLT Low reactive level laser therapy Mitochondria Trigeminal nerve Bradykinin Diode laser

Introduction

The use of low reactive level laser therapy (LLLT) for pain attenuation has appeared frequently in the literature.^{1,2} There are many hypotheses regarding the mechanism or pathways involved,²⁻⁴ but few have yet been morphologically examined and proved. In this experiment, bradykinin was used as a pain-producing agent, subcutaneously injected into the trigeminal dermatome of the Wistar rat. One group of animals was additionally irradiated with a gallium aluminium arsenide (GaAlAs) diode laser. A histological examination was made of the irradiated nerve fibres. The trigeminal nerve was chosen as the experimental injection site because its dermatome is more easily identifiable than that of other spinal segments.

When bradykinin is injected into the trigeminal dermatome,⁵ the resultant nerve impulses are conducted along the C-fibres, passing through the trigeminal ganglion and reaching the spinal trigeminal nucleus of the caudal medulla oblongata. In this area, the afferent terminal consists of synaptic

glomeruli, which are thought to play an important role in the transmission of nociceptive nerve impulses from the orofacial region in both man and the rat.

Materials and Methods

The Laser System

The laser used was a GaAlAs diode laser system, the OhLase-3D1 developed by the Japan Medical Laser Laboratory, Tokyo. The system consists of a control console, from which the output power and irradiation time are controlled; elapsed time is indicated on a digital display. The console is connected to a hand-held probe by means of a flexible connecting cable. The probe contains three laser diodes which are focused together to a common focal waist. The system operates in the near infrared at 830 nm, with an output power of 60 mW continuous wave, and a spot size of approximately 2 mm. This gives a power density at the focal point of approximately 1.9 W/cm².

Method

Three groups (A, B and C) of mature male Wistar strain rats weighing 250 g–300 g were used, six animals in each group. The animals were anaesthetized with ether. Group A received bradykinin alone, (Sigma) 1 mg/kg dissolved in physiological saline solution (0.4 ml/kg). The solution was injected subcutaneously in the maxillary region, twice daily for four consecutive days. Group B, following the bradykinin course, were irradiated with the diode laser at 12 points over the entire trigeminal dermatome, 15 s per point, twice per day for seven days. Group C was used as a control, receiving only physiological saline injections (0.4 mg/kg) as described above for groups A and B (Table 1). The method of treatment in this study followed the method for capsaicin treatment, a drug which appears to act selectively on C-fibres.⁶

Morphological observations were made on the 12th experimental day. The animals were anaesthetized with sodium pentobarbital (50 mg/kg), and perfused with a fixative containing 2.5 per cent glutaraldehyde, 2 per cent paraformaldehyde and 2 per cent picric acid in 0.1 M phosphate buffer, pH 7.3, for 30 min at room temperature. The medulla oblongata at the obex level and the trigeminal ganglion on the treated side were removed. The materials were rinsed for several hours in 0.1 M phosphate buffer (pH 7.3) containing 8 per cent sucrose, and were then sectioned into 100 µm slices with a microslicer.

The specimens were postfixed in 1 per cent OsO₄ for 2 h, dehydrated through a graded series of alcohols, and embedded in Spurr's resin. Ultrathin sections were made using a Reichert Ultracut E, and double-stained with uranyl acetate and lead citrate, and examined under a JEM 2000 EX(JEOL) electron microscope at 80 kV.

In addition, for the quantitative analysis, seven randomly chosen electron micrographs of each portion were taken at a magnification of × 20 000. The volume of mitochondria was estimated using a multipurpose test system containing 168 test points spaced at 2.0 cm on each photomicrograph, according to the point counting method.⁷

Results

Low density large nerve cells and high density small nerve cells were observed in the trigeminal ganglion. The number of mitochondria in the small pain-related ganglion cells treated with bradykinin only was noticeable higher than the others (Figure 1A). As for other organelles, enlarged lysosomes were also observed, but not as significant as the increase in mitochondria.

Table 1. Treatment schedule for rat groups

A	Bradykinin	
B	Bradykinin	111.T irradiation
C	Physiological saline

Figure 1. Photomicrographs of trigeminal ganglia, animal groups A and B. (A): Group A (bradykinin only group) × 10 000. (B): Group B (bradykinin plus 111.T with diode laser) × 7500. Note the marked decrease in the cytoplasmic mitochondrial volume of (B) compared with (A)

The axioms originating from the trigeminal ganglion terminate at the gelatinous substance of the caudal spinal trigeminal nucleus, forming synaptic complexes. The axons originating from the small ganglion cells are situated in the centre of the synaptic complex, and demonstrate a morphologically higher electron density than the axons of the large ganglion cells. It was observed that the mitochondrial density of the axon terminals increased following bradykinin treatment (Figure 2A). However, the mitochondrial density in both the small ganglion cells of the trigeminal ganglion and in the axon terminals of the spinal trigeminal ganglion caudal nucleus decreased following diode laser irradiation (Figures 1B, 2B). There were no clear morphological differences between group B and control group C specimens. Figure 1 shows a typical electron micrograph of a trigeminal ganglion, and Figure 2 shows a spinal trigeminal nucleus. The

Figure 2. Photomicrographs of the spinal trigeminal nucleus of the caudal medulla oblongata in groups A and B. (A): Group A (bradykinin only) $\times 40\,000$. (B): Group B (bradykinin plus LLLT with diode laser) $\times 46\,000$. Mitochondrial volume is again clearly less in the bradykinin plus laser-treated group

statistical data derived from the point counting method using these photomicrographs is shown in Table 2. Statistical analysis by the student's *t*-test showed a significant difference in the increase in mitochondrial density between groups A and B ($P < 0.001$). It was thus indirectly demonstrated that one of the mechanisms of laser pain attenuation is to reduce the peripheral nerve impulses caused by bradykinin.

Discussion

The increased mitochondrial density following bradykinin treatment is induced by increased nerve impulses. Bradykinin is a naturally-occurring bodily substance,⁵ consisting of nine amino acids. It is one of the strongest pain-producing agents. There are

Table 2. Mitochondrial density of rat groups

Group	Number	Small ganglion cell	Axon terminal
A	6	0.592 ± 0.087	0.684 ± 0.092
B	6	0.264 ± 0.051	0.186 ± 0.064
C	6	0.221 ± 0.037	0.146 ± 0.068

two main skin pain sensations: a sharp, short acute pain, and a dull, burning pain of long duration. Bradykinin produces the latter pain type, affecting sensitive free nerve endings, causing an increase in the number of nerve impulses. When infiltrated with high-density bradykinin, nerve cells show a significant increase in the number of mitochondria in the cytoplasm. Lysosomes also increase, but not to such a great extent.

The mitochondrion is a cellular organelle which synthesizes high energy phosphates such as ATP, using the TCA and beta oxidation.⁸ Mitochondria also contain autoreplicating DNA, enabling cell multiplication by fission. It is already recognized that there is an increase in mitochondrial volume concurrent with an increase in ATP consumption; it could therefore be said that an increase in ATP consumption is a trigger for mitochondrial proliferation. Nerve impulses are accompanied by an internal flow of sodium and an external flow of potassium. In restoration, active transport operates (the Na-K pump), causing an increase in ATP consumption. ATP is also consumed in the synaptic glomeruli to emit neurotransmitters, and the axon transport of the neurotransmitter after synthesis in the pericaryon also consumes ATP. When there is a higher rate of neural impulses over a prolonged period, the existing mitochondria cannot supply sufficient ATP to fuel the increased neuron activity. Accordingly, a secondary mitochondrial proliferation occurs. Following LLLT irradiation, the volume of mitochondria in the bradykinin-affected cells decreased. It is known that LLLT increases the local blood supply.^{9, 10} It can be assumed that the increased blood flow will absorb excess bradykinin from the affected area, thereby decreasing the bradykinin density, and thus lowering the level of neural activity. This naturally lowers the ATP consumption, and so the mitochondrial volume returns to normal.

In a previous study, nerve fibre Na-K-ATPase activity after LLLT irradiation was investigated.¹¹ It was discovered that shorter irradiation times increased the activity level, and longer times decreased it. It could therefore be presumed that the longer LLLT doses in the present study had a similar effect on the Na-K-ATPase activity level in the trigeminal nerve fibres, leading to a decrease in the number of mitochondria as the demand for ATP decreased.

From this study, another possible mechanism of pain attenuation following LLLT irradiation has been morphologically demonstrated.

Acknowledgements

It is at present difficult to detect pain-related nerve impulses by electrophysiology alone. Accordingly the above method

morphological technique for measuring mitochondrial density as an indication of increased or decreased impulses was developed by the Second Department of Anatomy, School of Medicine, Toho University. The author wishes to acknowledge the assistance of Professor Kenichiroh Inomata MD DMSc, Toshio Ohshiro MD, Fumio Nasu MD, and Chiaki Kudoh MD for their invaluable assistance with this research.

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