Improved Regeneration of Injured Sciatic Nerve of Rats by He-Ne Laser

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Inability of the neural tissue to regenerate in response to trauma and the disability which the result has prompted a lot of effort in search for new means of neural rehabilitation. Low-energy Helium-Neon laser (He-Ne laser) irradiation has been proposed as a sensible choice. Low-energy laser therapy in specific wavelengths and affluence maintains the electrophysiological activity of injured peripheral nerve in rat and accelerating regeneration of the injured nerve. We studied the effect of low level laser irradiation (He-Ne laser) on regeneration of injured sciatic nerve of rats. In the current experiment, sciatic nerves of 20 adult rats were crushed surgically. The subjects were randomly assigned into case and control groups. The cases received daily laser irradiation (λ = 650 nm) for 4 min. The muscular function of the rats was tested by angle board each 3 days, since the 3rd day of post operation. On the 27th day, all the rats were sacrificed and the manipulated sciatic nerves were excised and studied histologically. The results showed significant improvement of neural structure and muscular function in laser treated rats over the controls, as revealed by angle board testing and microscopic examination. It seems that low-energy lasers can reduce the load of infection at the operation field, tissue inflammation and post operation pain. This study show that low-energy He-Ne laser greatly restores crushed sciatic nerves in rats and can be considered for clinical trails.

Key words: Helium-Neon laser (He-Ne laser), sciatic nerve, muscular function, angle board, microscopic examination, rat
INTRODUCTION

Low level laser therapy is reported to influence soft tissue repair (Walker et al., 2000) and nerve regeneration (Rochkind et al., 1987a, b, 1989, 2001) and has therefore been used to treat a wide range of etiological diseases. Neuronal tissue is one of the most important tissues of the body which recovers extremely sluggishly after being injured (Walker et al., 2000; Rochkind et al., 1987a, b, 1989, 2001a, b; Nicolau et al., 2004). After the primary degeneration in response to trauma, its function is restored slowly and only the neural fibers are regenerated and have come in contact with the target organ (Rochkind et al., 2001 a, b; Nicolau et al., 2004; Samoilo, 1991). The reason is the central nervous system has restricted regenerative capability. The medical researchers have been constantly endeavoring to find practical ways to restore the injured nerves (Walker et al., 2000; Rochkind et al., 2001; Nicolau et al., 2004; Nissan et al., 1986). Therefor, there have been some invasive procedures such as surgical methods and non-invasive ones like physiotherapy. Despite all efforts, heavy costs and the long time which it takes, the result is hardly more than slow neural regeneration and a doubtful outcome and the repaired nerve rarely resumes its optimal function. The application of low-energy lasers, especially He-Ne type, has opened up new windows of hope in neural injuries (Rochkind et al., 1987a, b, 1989, 2001; Samoilo, 1991; Andres et al., 1993; Rochkind, 1992; Midamba and Haanias, 1993; Ijima et al., 1991). This method is absolutely non-invasive and its exclusive features have rendered it a relatively unique stance among all other methods (Rochkindw et al., 1987; Schwartz et al., 2002; Jacobs and Fehling, 2003). Currently, low-energy lasers are widely used for neuromuscular lesions from dermatologic, rheumatologic and neurosurgical conditions. Low-energy lasers do not change the tissue temperature immediately but manipulate biological processes so their effect takes time to show (Mester, 1996; Babapour and Classbory, 1998). Today, many researchers have turned to this type of lasers and their clinical application is appraised (Passarella and Gasomassina, 1984; Miloro et al., 2002). Experimentally, low-energy lasers have significantly helped regenerating the peripheral nerves and increasing the axonal density of the injured nerves after trauma and iatrogenic etiologies (Rochkind et al., 2001a, b; Bages et al., 2002, Shamir et al., 2001). In this study, we investigate the effect of He-Ne laser in regeneration of injured sciatic nerve of rats by simple method.

MATERIALS AND METHODS

Experimental procedure: This case-control study was performed on 20 Wister-albino rats (weighing 200-250 g). This strain used for many years in our laboratory in Tehran University, of medical science (2005-2006). They were kept in individual cages in a controlled room (temperature, 20-25°C humidity, 70 to 80%, exposed to 12 h of daylight). The rats were fed with standard rat food and tap water until experimentation. Twelve hours before the experiment the rats were stopped feeding but allowed free access to tap water. Limitation of food and water was not applied to the animals that were put into their cages after experimentation and were kept in separate standard cages under sterile conditions and the ambient air was kept at 25-30°C. All experiments were conducted in Tehran University according with the recommendations of the ethics committee on animal’s experimentation of medical school.

Denervation: On the day of procedure, the operation area on the rat’s skin was shaved and cleansed with Povidone-Iodine. Then the animal was anesthetized by ketamine. Under sterile conditions, an oblique incision was made in the paravertebral area near the hind legs just above the sciatic nerve and the skin and muscular fascia were carefully opened. Then the sciatic nerve was exposed and fixed by two pairs of forceps. Manual pressure was applied to the middle of the fixed part of the nerve, constantly for 90 sec using hemostats. Then traumatic nerve was replaced and the incision sutured (Rochkind et al., 2001a, b; Yueh et al., 2005). This procedure was performed on all the rats and the animals were randomly assigned into case and control groups.

Laser irradiation: Over a 27-day period on a daily basis, the rats in the case group were irradiated by low-energy He-Ne laser, at a wavelength (λ) of 650 nm, for 4 min.

Muscle function testing: Every 3 days since the 3rd day after the procedure, the rats of the case and control groups were tested for muscle force and coordination by means of a Riveline-Tatore angle board. After 27 days, all the rats were sacrificed and the traumatic portions of their sciatic nerves were excised for histological examination.

Statistical analysis: Values are expressed as Mean±Standard Error of the Mean (SEM) in tables. Statistical significance was evaluated by two-tailed student’s t-test. The significance was set at p<0.001.

RESULTS AND DISCUSSION

The data collected from the angle board testing is shown in Table 1. This test is a simple method for assessing the function of sciatic nerve indirectly from the muscle power and coordination of the animal's hind legs.
Table 1: Mean muscle force of cases and controls on angle board
(Comparative results between cases and controls on different day)

<table>
<thead>
<tr>
<th>Days</th>
<th>n</th>
<th>Control group</th>
<th>Case group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>10</td>
<td>1.34±0.68</td>
<td>4.5±0.69</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>7.2±0.69</td>
<td>12.5±0.78</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>11.3±0.68</td>
<td>16.2±0.88</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>16.9±0.72</td>
<td>24.7±0.93</td>
</tr>
<tr>
<td>18</td>
<td>10</td>
<td>27.7±0.79</td>
<td>35.2±1.61</td>
</tr>
<tr>
<td>21</td>
<td>10</td>
<td>35.5±0.75</td>
<td>42.5±1.34</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>35.1±0.90</td>
<td>48.2±1.39</td>
</tr>
<tr>
<td>27</td>
<td>10</td>
<td>41.3±1.01</td>
<td>55.5±1.16</td>
</tr>
</tbody>
</table>

There was significant increase in cases groups compared with control groups in muscle force on angle board test.

Table 2: Diameter of myocytes in cases and controls

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Mean diameter of myocytes±SEM</th>
</tr>
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<tbody>
<tr>
<td>Case</td>
<td>10</td>
<td>2.26±0.59</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>1.72±0.66</td>
</tr>
</tbody>
</table>

There was significant increase in cases groups compared with control groups in size of myocyte.

As peripheral nerve lesions affects the pertinent tissues, leading to muscular atrophy and weakness, showed by microscopic changes in the target myocytes. This difference between in the cases and controls was compared in Table 2. The t-test analysis showed that the difference between the angle board results of cases and controls were statistically significant (p<0.001).

Low-energy laser acts as a focused source of energy and stimulates both the neurons and bystander cells. Low-energy laser irradiation in pain reduction has been observed in neurological studies. Recent studies have been suggested that the low-energy laser can improve recovery from nerve injuries in both rats and humans (Nicolau et al., 2004; Nissan et al., 1986; Samoilov, 1991; Andres et al., 1993; Rochkind, 1992, Midamba and Haaneas, 1993; Ijima et al., 1991). Mester and Mester (1996) reported the noticeable effects of low-energy laser irradiation on biological systems. It be demonstrated the regulatory role of laser beams on restriction of the primary inflammatory response (Bendov et al., 1999) and enhanced the healing of the injured tissue (Milorer et al., 2002; Rochkind et al., 2001a, b). The present experiment reproduced similar results revealing the significant difference between the neural regeneration in cases and controls. The microscopic specimens of the case group which received periodical laser irradiation clearly showed laser had prevented degeneration of the nerves and accelerated regenerative processes. Low-energy laser first introduced as effectiveness in biological systems (Mester and Mester, 1996). Studies have shown that certain wavelengths and energy densities of low-energy lasers can revive the neuronal function of the injured peripheral nerves, diminish degenerative changes in sensory and motor neurons and enhance the regeneration of traumatic nervous tissues (Bages et al., 2002, Shamir et al., 2001; Kimberly et al., 2005; Dong et al., 2003). Upon microscopic examination of the laser irradiated sciatic nerves, it was observed that the neural fibers had resumed their normal diameter and their Schwann cells and myelin coating were preserved. There were fewer macrophages as compared with the control group, which demonstrate reduced inflammatory reaction at the site of trauma. The muscular fibers with the injured sciatic nerves did not show the signs of atrophy when treated by laser, appeared normal diameter in size and color of the sarcoplasm. The function of muscles were improved as well as the control group which demonstrated by the angle board test results. The natural course of neural damage after the traumatic insult is observed in the control group. Primarily, the myelin sheath is fragmented into pieces to be taken up and digested by macrophages. As seen in the control group of rats, the degenerative and following regenerative changes are depicted by the numerous macrophages and Schwann cells. The natural consequence of neural deprivation of muscles was atrophic changes. The atrophic changes and lack of muscular function early after the operation in the control group of rats demonstrated in this Table 2.

This finding showed atrophic and microscopic changes in muscles and myocytes in the cases and controls similar to previous studies (Nicolau et al., 2004; Nissan et al., 1986; Samoilov, 1991; Andres et al., 1993; Rochkind, 1992, Midamba and Haaneas, 1993; Ijima et al., 1991; Mester and Mester, 1996). Present result suggests that low-energy lasers can reduce the load of infection at the operation field, tissue inflammation and post operation pain. As a condensed source of energy, it manipulates the cellular metabolic processes. Since it is a harmless vital stimulant, we recommend that He-Ne laser therapy be clinically applied after necessary clinical trials and considered as a very promising, Physiotherapeutic tool.

REFERENCES


