THE EXPRESSION OF TGF-β1 AFTER LOW LEVEL LASER THERAPY IN INFLAMMATION ANIMAL MODEL

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ABSTRACT

Pain is the most common complaints caused by stimuli due to potential tissue damage. Low level laser therapy (LLLT) has been used in reducing pain. One of indicator tissue healing is activation of transforming growth factor-β1 (TGF-β1). Tissue healing will eliminate the pain. Do LLLT stimulate TGF-β1 expression? The objective of this study was to investigate the expression of TGF-β1 in low level laser therapy. Thirty male Wistar rats, body weight 250 – 300 gram, 3 month old, were divided into 3 groups, negative control (n=10), positive control (n=10) and treatment groups (n=10). The positive control and treatment groups were injected with Complete’s Freund Adjuvant (CFA) 100 μL at telapak kaki kiri. Sample dikelompokkan menjadi tiga kelompok yaitu K0 (n=10) tikus normal; K1 (n=10) tikus yang diinduksi CFA saja; P1 (n=10) tikus yang diinduksi CFA ditambah terapi laser berkekuatan rendah dengan Lasermed tipe 2100 dengan panjang gelombang 905 nm, out put power 25 – 500 mW power: 30 mW/cm2, dosis: 1 Joule/cm2; 5 kali dengan interval 24 jam pada telapak kaki kiri. Sampel diambil dari kulit telapak kaki kiri dan dilakukan pemeriksaan immunohistokimia dengan antibodi monoclonal anti TGF-β1. Penghitungan dilakukan terhadap sel yang imunoreaktif tercat coklat pada sel makrofag. Data dianalisis menggunakan uji Games Howell dengan perangkat lunak SPSS 17. Terdapat perbedaan yang signifikan ekspresi TGF-β1 pada kelompok terapi laser berkekuatan rendah (15.8±3.1) terhadap kelompok kontrol positif (3.9±1.1) dan terhadap kelompok kontrol negatif (4.6±2.1), p<0.05. Tidak ada perbedaan yang bermakna antara kelompok kontrol negatif dan kelompok kontrol positif. Disimpulkan bahwa terapi laser berkekuatan rendah meningkatkan ekspresi TGF-β1. (FMI 2017;53:29-32)

Keywords: Low level laser therapy, TGF-β1

INTRODUCTION

Pain is the most complaint in everyday practice. The prevalence of chronic pain in Europe was 19% (Breivik, 2006), United State 30.7% (Johannes, 2010), while in Hong Kong 34.9% (Wong, 2011). According WHO, the prevalence of chronic pain in developing countries 41% (Croft, 2010). Pain restrict activities daily living and cause disabilities. The economical impact of pain due to loss of productive time, was estimated 61.2 billion dollars annually (Reyes-Gibby, 2008). Reducing pain as soon as possible is very important. The most common treatment used for pain relief currently is non-steroid anti-inflammatory drugs (NSAIDs) (Buvanendran,
2010), but NSAIDs caused serious effect such as gastric bleeding (Fujimori, 2010). Besides drugs, pain can be attenuate by heat therapy, water therapy and electrical therapy but all the treatment are still not satisfactory. Lately, low level laser therapy (LLLT) effectively reduce pain. Hegedus (2009) reported that LLLT reduced pain and improved microcirculation in patients with knee osteoarthritis. The hypothesis proposed that photon emitted LLLT captured mainly in mitochondrial cytochrome c oxidase causing a cascade cause biostimulation cell (Avci, 2013). In previous study, we found that LLLT increased HSP 70 and opioid receptor expression. The expression of opioid receptor inhibit neurotransmitter glutamate, so reduced pain.

Tissue repair is an interactive process, involving chemical mediators, cells, and the inflammatory response, characterized by the classic steps of repair: inflammation, granulation, and remodeling. The clinical efficacy of LLLT in wound healing has been reported. It has been found to significantly decrease area of wound healing (Kajagar, 2012). In proliferation phase in wound healing, transforming growth factor–β1 (TGF-β1) play an important role (Amid, 2014). TGF-β1 acts as a regulator of growth and differentiation of cells, as well as playing an important role in the formation of the extracellular matrix (Colombo, 2013). Cell studies with cultured human keratinocytes, endothelial cells, and fibroblast indicated potential effects of near-infrared light in the treatment pf chronic skin ulcers. After irradiation of cells, the production of transforming growth factor β1 (TGF-β1) and matrix metalloproteinase-2 (MMP-2) was examined by immunohassay, zymography, and reverse transcription polymerase chain reaction (PCR). A biostimulatory effect of near-infrared irradiation was shown by significant elevation of TGF-β1and MMP-2. Irradiated fibroblast also showed an upregulated amount of MMP-2 mRNA. These results suggest that near-infrared irradiation may accelerate wound closure (Beckman, 2014). In this study, we used an inflammatory pain animal model to know the expression TGF-β1 during inflammatory response.

MATERIALS AND METHODS

The present study is a randomized post test control group design. Adult male Wistar rats weighing 250–300 g. 3 month old were purchased from Department of Farmacology Faculty of Medicine Universitas Airlangga. There were housed 4-5 per cage with food pellets and water ad libitum for 7 days after their purchase, thereafter they were used for experiments. During the entire study, all rats were kept in an environment with a cycle of 12 h light and 12 dark. Rats were kept at room temperature (22±10C). Study protocol has been approved by ethic committee of Faculty of Veterinary Medicine, UniversitasAirlangga, Surabaya, Indonesia (Ethics No. 504-KE).

In order to know the LLLT effects, 30 rats were randomly divided into three groups: negative control group (without any intervension), positive control group (CFA only) and treatment group. 100 µL CFA was injected into the ventral surface of the left foot centered in the footpad of rats in positive control group and treatment group. In treatment group, 48 hours after CFA’s injection treated with low level laser therapy Lasermed type 2100 wavelength 905 nm, out power 25-500 mW, dose 1 joule/cm2 for 5 times with interval 24 hours.

The expression of TGF-β1 were tested by immunohistochemistry method using antibody monoclonal anti TGF-β1. Macrophages that produce brown color against monoclonal antibody anti TGF-β1 are positive reaction. Positive cells for TGF-β1expressions were counted by using light microscope (Olympus CX21, New York, USA) at 20 microscopic fields of 1000 magnification. Kolmogorov-Smirnov test was used to check normality of all data. All data were normally distributed (p>0.05). Homogeneity data with Levene’s test showed that TGF-β1 data were not homogen. Therefore Games-Howell was used to compare between negative control, positive control and treatment group for the expression of TGF-β1. Statistic package SPSS 17 was used.

RESULTS

Immunohistochemical staining showed elevated numbers of macrophage give a brown color reaction against anti-TGF-β1 antibody subjected to LLLT, compared with the positive control groups (figure 1).

Table 1. Number of macrophages that expressed anti TGF-β1

<table>
<thead>
<tr>
<th>Groups</th>
<th>TGF-β1</th>
<th>Games Howell (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SD</td>
</tr>
<tr>
<td>Negative control</td>
<td>4.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Positive control</td>
<td>3.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Treatment</td>
<td>15.8</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Table 1 showed that there were no significant different between negative control group and positive control group. There were significant different between positive control group and treatment group. These result showed that LLLT enhance the expression of TGF-β1.
The Expression of TGF-β1 after Low Level Laser Therapy in Inflammation Animal Model (Imam Subadi et al)

DISCUSSION

In the field of medical rehabilitation, low level laser therapy was used to reduce pain. According to the International Association for the Study of Pain (IASP) pain is defined as the experience of sensation and emotional discomfort associated with apparent tissue damage or potential damage or described in terms of damage (Turk and Okifuji, 2010; King and Dougherty, 2011). Animal used to inflammatory pain model was rats induced by CFA (Zhang, 2011). This LLLT was gallium arsenide with a wavelength of 830 nm, a dose of 1 Joule/cm2. In the last study we found the expression of HSP 70 and mu opioid receptors (Subadi I, 2014). Induction CFA in experimental animal cause inflammation. In the inflammatory phase of a healing process increase reactive oxygen species (ROS) and activate of NFkB expression, a transcription factor that is responsible for pro-inflammatory mediators such as interleukin and TNF-α, growth factors, chemokines and adhesion molecules (Assiss, 2012). One of the growth factors, namely TGF-β plays an important role in the proliferation and migration of fibroblasts, and increasing collagen synthesis and breakdown of extracellular matrix. On the other hand, a LLLT is effective in reducing inflammation and decreased the expression of IL-1β, IL-6 and TNF-α (Alves, 2013). TGF-β1 was the most responsible for proliferation phase.

In this study, LLLT enhance TGF-β1 in the treatment group (15.8±3.1) compared to the positive controls (3.9±1.1) and negative controls (4.6±2, 1). There was no differences between positif and negative control group. It can be concluded that the inflammatory process induced by CFA did not cause the expression of TGF-β1 and LLLT significantly increased the expression of TGF-β1. The weakness of this study is the small sample. Saafan et al (2013) using a low power laser diode (LPDL) with a wavelength of 810 nm, 200 mW power output, duration of therapy to 80 seconds, the power density of 0.2 W/cm2, the energy density of 16 J/cm2, gain increased TGF-β1. Colombo (2013) using a laser wavelength of 660 nm, 16 mW 10 J/cm2, do not get increased expression of TGF-β1. Humblin et al (2006) report that administration of a low power laser therapy led to increased production of growth factors TGF-β1 that responsible for inducing the synthesis of collagen from fibroblasts. Increased synthesis of TGF-β are important in tissue repair. Beckmann et al (2014) conducted a systematic literature review of the 1764 article and concluded that in studies with cell and animal shows cell migration, fibroblast proliferation, rapid formation of new tissue, increased microcirculation and anti-inflammatory effect by inhibition of prostaglandin, interleukins and cytokines and bacterial effect by inducing reactive oxygen species (ROS) after LLLT. The majority of studies show benefit for diabetic wound healing.

CONCLUSION

Low level laser therapy stimulate the expression of TGF-β1 on an animal model of inflammation pain.

REFERENCES


