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The optimal dose of the low-level laser therapy in the treatment of type 1 diabetes mellitus



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Abdurachman,^{1,2*} Rubiyanto, A.,³ Suhariningsih,⁴ Hendromartono,⁵ Gunawan, A.⁶

ABSTRACT

Background: Type 1 DM (T1DM) is caused by an autoimmune destruction of pancreatic β cells. We proposed a novel stimulation using a low-level laser therapy (LLLT) through acupuncture points to stimulate the stem cells to be adult pancreatic β cells. This study aimed to identify an optimal dose of low-level laser therapy (LLLT) for treatment of type 1 diabetes mellitus (T1DM).

Methods: An experimental study was performed with a pretest-posttest control group design. The intervention was tested in rats (*Rattus norvegicus*, Wistar strain). The rats were made diabetic by injection of streptozotocin (STZ). Rats were treated with different doses of LLLT in 4 groups. The tested laser doses were 0.2 joules (group L1), 0.3 joules (group L2), 0.4 joules (group L3), and 0.5 joules (group L4).

The therapy was performed by perpendicularly attaching laser probe to the animal body, at the acupuncture point pishu (BL-20) on respective sides.

Results: Rats in the L2 group were performing better compared to others. The highest decrease of blood glucose level was found in group L2. Two rats in the L2 group achieved normal blood glucose level upon laser therapy. The dose of laser therapy in the L2 group of 0.3 joules was found to be the optimum dose.

Conclusion: Clinical and statistical analyses indicated that 0.3 joule was the optimal dose for LLLT in our animal model. Further investigation of LLLT in human is warranted, and validation of the identified dose in future studies should be pursued.

Keywords: Low-level laser therapy, type 1 DM, optimal dose

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease which can lead to multi-organ failures. The number of people with DM worldwide is currently increasing. It was reported in 1994 that the number of individuals with DM around the world was 110.4 million. This figure, however, has grown steadily in 1998, 150 million; in 2000, 175.4 million; in 2010, 279.3 million, and in 2020, it is estimated to be 300 million.¹

Type 1 DM (T1DM) is caused by an autoimmune destruction of pancreatic β cells. Although T1DM has been described since the beginning of ancient Egyptian civilization, the disease is still a leading cause of death until the beginning of the 20th century.² Researchers around the world have performed numerous efforts to try to overcome this problem. Recent evidence has shown some success of Langerhans islet transplantation performed in several centers worldwide.³ β cell metabolism pathway can directly utilize extracellular glucose, thus making it readily secrete insulin to allow blood glucose level reaches its normal value.⁴ Ideally, the newly transplanted β cells should similarly perform as normal upon transplantation. However, this has been proven to be challenging in the actual clinical settings.

Another way to address the issue is by the utilization of stem cells. Stem cells in an adult human

have been found in liver, brain, skin, bone marrow, intestinal epithelium, and heart muscle, hence it is thought to be present in the pancreas as well.^{5,6,7,8,9,10} Stem cells, however, also have several limitations. They are only able to turn into the desired mature cells depending on their location. Special stimuli are needed to allow differentiation into mature cells, which are thought to be based on their location. It should be occurring in such an environment in which the stem cells could receive certain signals to allow them to undergo reprogramming and continue differentiation according to the received information. To date, it has been shown to be difficult to characterize the specific trigger to allow differentiation of the cells into mature cells within a similar function of the destroyed pancreatic cells. Not much in this context are known regarding the mechanism of changes of stem cells in adult human.

Scientists have been trying to provide the right stimuli so that the stem cells which are initially in a “resting” phase can be triggered to differentiate towards mature adult cells.^{2,8,9,10} One of the first efforts was performed by Li et al.¹¹ The group used Betacellulin to stimulate the stem cells to differentiate into β cells. The research was performed on male Wistar rats which had undergone pancreatectomy.

¹Department of Anatomy and Histology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

²Traditional Medicine Study Program (BATTRA), Faculty of Vocational Studies, Universitas Airlangga, Surabaya, Indonesia

³Professor, Ph.D., Optoelectronics Medical Physics and Biophysics, Institut Teknologi Sepuluh Nopember (ITS), Surabaya, Indonesia

⁴Professor, Ph.D., Department of Biophysics, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia, suhariningsih@unair.ac.id

⁵Professor, Ph.D., Department of Internal Medicine (Indonesia Center for Diabetes Mellitus Study), Dr. Soetomo Teaching Hospital-School of Medicine, Universitas Airlangga, Surabaya, Indonesia.

⁶Professor, Ph.D. Department of Anatomy and Histology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

*Correspondence to: Abdurachman abdurachman@fk.unair.ac.id; abdurachman1166@gmail.com

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Another observation conducted by Waguri et al.¹² managed to provide some evidence regarding the characteristics of pancreatic β cells to possess an ability of self-regeneration. Regeneration of damaged cells (self-repair) was described as the generation of new β cells from the epithelial cells of the pancreatic duct lining. With an appropriate stimulus, regeneration was shown to begin within 48 hours, while the completed process was achieved within 10-11 days.¹³

In this study, previous findings as discussed above will be further investigated with a treatment technique that uses stimulation methods. Stimulation performed at certain points is expected to provide a therapeutic effect and is the basis of the present acupuncture therapy. For the treatment of T1DM, according to the current technique, the selected point for acupuncture is the BL-20, back-shu which represents the pancreas.^{14,15,16,17,18} Such stimulation has been commonly performed with acupuncture needles.¹⁹ In this study, we proposed a novel stimulation using a low-level laser therapy (LLLT). Laser therapy has many advantages compared to the use of needles.²⁰ Laser doses option for LLLT which has been previously identified were 0.2 joules, 0.3 joules, 0.4 joules, and 0.5 joules.²¹ In this study, we aimed to identify an optimal dose of laser for future therapy of T1DM using an animal model.

METHODS

In this experiment, the rats used were male *Rattus norvegicus* (Wistar strain), 126 - 167 gram of body weight (BW), aged three months. All animals were kept in cages at a temperature of 20 - 25°C. Time of day and night were divided evenly, each 12 hours. Daylight was defined as the time between 6 am to 6 pm, while the evening was from 6 pm to 6 am. All animal work was carried out following the Faculty of Medicine Airlangga University (FMAU) institutional guideline.

Streptozotocin (STZ) was injected intraperitoneal to create an animal model of T1DM. The dose of STZ used was 60 mg/kg,^{22,23} prepared in a solution of 0.1M citrate buffer (pH = 4.5). We

used a low pH of STZ solution so that the active substance was not easily inactivated. We obtained STZ from Merck Tbk, Chemical division (Catalog no. 572201-1GM, batch B56981). Measurement of fasting blood glucose levels was performed 48 hours after the injection with STZ.²⁴ Blood glucose level measurement was done using a Johnson-Johnson One-Touch Strip test (Lifescan Inc., a Johnson & Johnson company, Milpitas, CA 95035 U.S.A. No. SMC4212QT). In this experiment, the cut-off level for T1DM in rats was 300 mg/dl.²⁴

Once the animals have been T1DM-induced, 16 test animals were randomly divided into four groups. Each group consisted of 4 animals. Two days after the injection with STZ, blood glucose levels were measured to ensure all rats were T1DM. Afterwards, each group received laser therapy on BL-20 acupuncture points on both sides (right and left). The dosage of the laser used for group I (L1) was 0.2 joules, group II (L2) 0.3 joules, group III (L3) 0.4 joules, and group IV (L4) 0.5 joules. The laser therapy was given every two days, six times consecutively for 12 days starting in 24 hours after the rats have been T1DM-induced.²⁴

Laser therapy was performed using a type of semiconductor laser Aluminium-Gallium-Indium-Phosphorous (Al Ga In P), which has a wavelength of 650 nm. Laser probes were attached to the body of rats.^{25,26,27} The laser light was perpendicularly directed to the bilateral BL-20-point. Fasting blood glucose levels were measured 13 days after the start of therapy. The blood samples were obtained from the tail of the rat. The pancreas of the rats was removed immediately after the rats were sacrificed. The rat conditions were observed every day.

RESULTS

The daily observation suggested that the rats in the L2 group were in general performing better than others. Rat hairs in the L2 group were thick and clean, intake of food was good, activities were better, and body weight tended to improve. There were two rats in the L2 group which had blood glucose level below

Table 1 Blood glucose levels before and after treatment

Group	N	Pretest		Posttest		p	The decrease	
		Mean	SD	Mean	SD		Mean	SD
L1	4	600.00	0.00	482.00	103.32	0.055	118.00	103.32
L2	4	577.25	40.34	294.25	113.84	0.034*	283.00	132.80
L3	4	578.50	41.68	389.75	11.33	0.034*	188.75	42.57
L4	4	500.25	59.21	312.50	24.77	0.034*	187.38	44.09
P		0.039*		0.017			0.130	

L1 = 0.2 joule LLLT, L2 = 0.3 joule LLLT, L3 = 0.4 joule LLLT, L4 = 0.5 joule LLLT

* = significant at $p < 0.05$

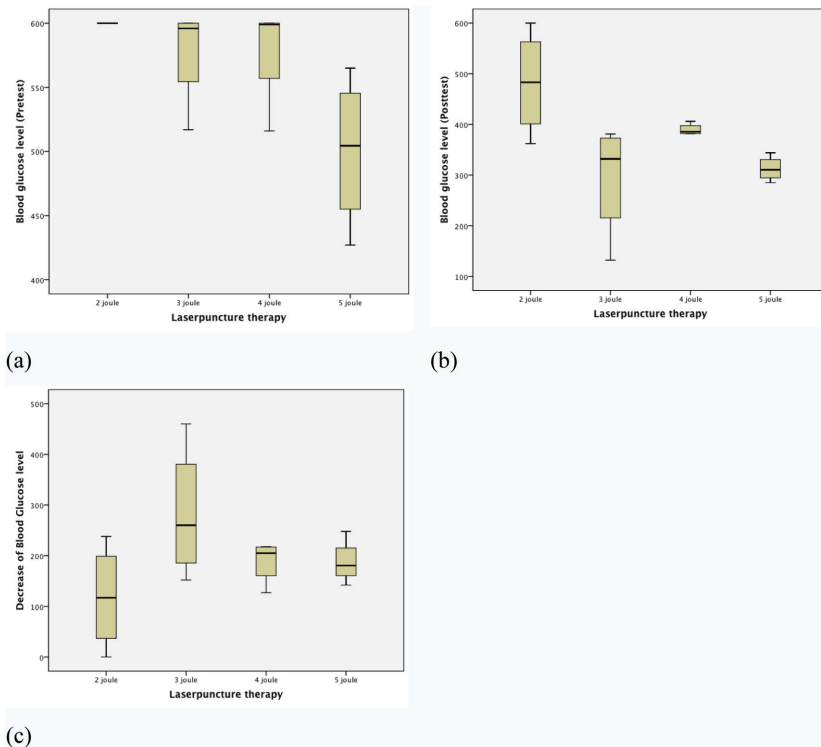


Figure 1 Average blood glucose levels in rats before treatment (a), after treatment (b), and the magnitude of decrease after treatment (c)

cut-off at the end of the study. The blood glucose levels were 299 mg/dl and 132 mg/dl, respectively.

Figure 1 (a) shows the initial condition of rat's blood glucose level before treatment. Blood glucose levels of rats in the L4 group were the lowest compared to other groups before treatment. While after therapy (b), rats in L2 and L3 groups had the lowest blood glucose levels. The highest decrease was found in group L2. Therefore, we concluded that the best result of the laser therapy was demonstrated in group L2. Rats in group L2 acquired laser therapy in the dose of 0.3 joules. Based on this finding, we might suggest the dose of 0.3 joules to be used in future studies.

DISCUSSION

Acupuncture Technique in Animal Models

The use of acupuncture needles has been shown to cause several problems in experimental animal models. One of the major problems that may arise is the pain. Pain may lead to significant changes in blood cortisol levels in animals and humans. Increased cortisol levels could affect the levels of circulating insulin; therefore, it will affect blood glucose levels. This change will disrupt the reading of the results and will give rise to dubious values, as previously described.^{25,26,27} To avoid these risks, a new acupuncture technique which does not use needles was selected. Such procedure does not cause pain which may cause stress in animals.

This technique was used to minimize the generation of dubious value and to achieve results with a high-precision significance.^{26,27}

Laser Puncture Techniques

The laser light for the therapeutic purpose should be used within its safety limit. Similar to the application of other physical interventions with the aim to obtain a healing effect, optimal doses should always be pursued. This study has demonstrated the optimal dose of laser light in an animal model. According to the first law of thermodynamics, the energy delivered to the tissue is conserved, and three possible pathways exist to account for what happens to the light energy when LLLT is delivered into tissue.^{25,26,27}

Laser Energy

The amount of energy dose received in the tissue is associated with intensity. Intensity (I) is defined as the amount of energy that penetrates the skin surface per unit area in a time unit. It can also be extrapolated as the quotient between the power of laser per area of skin which is exposed to laser energy (mW / cm^2) = Laser power (mW) / source per cross-sectional area (cm^2).

In a previous study by Suhariningsih et al. 2004²¹, a range of laser intensities was obtained in a clinical study enrolling 100 participants (Table 2). The study described an initial success of acupuncture therapy using the laser energy. Power (P) was expressed in W (Watts) or mW (milliwatt), meaning $1 \text{ W} = 1 \text{ J} / \text{s}$. In the study, the laser beam had an average power of approximately 3-100 mW, larger power was translated to a shorter duration of treatment. The magnitude of the average laser power used for acupuncture therapy based on the clinical study was described in Table 3.

The dose of energy (D) is the amount of laser light energy which is transferred to the body through acupuncture points during therapy. If the area of acupuncture points is quantified as A cm^2 and the laser output from the equipment is quantified as B cm^2 , the dose can be calculated as the function of equipment power, exposure time, and the comparison between A and B. The dose is expressed in J (joules).

$$\text{Dose (J)} = \text{Laser power (W or mW} \times 1000) \times \text{exposure time (sec)} \times \frac{A}{B}$$

A = area of acupuncture points, B = laser output
If B = A, meaning extensive laser light output of the equipment coincides with the acupuncture points, then:

$$\text{Dose (J)} = \text{Laser power (W or mW} \times 1000) \times \text{duration of exposure (seconds)}$$

$$\text{Or} \quad \text{exposure time/duration of therapy } t = \frac{D}{P} \text{ sec}$$

Table 2 Data distribution of laser light intensity

25-75 mW/cm ²	75-300 mW/cm ²	300-1000 mW/cm ²	> 1000 mW/cm ²
9%	45 %	27 %	19 %

*Adapted from Suhariningsih, 2004²¹

Table 3 Data distribution of laser power usage in acupuncture therapy

1-3 mW	3-10 Mw	10-30 mW	> 30 mW
9%	44 %	26.5 %	20.5 %

Adapted from Suhariningsih, 2004²¹

Table 4 Data on the dose distribution of laser usage

0.1-0.3 J	0.5-1 J	1-3 J	> 3 J
20 %	27 %	28 %	25 %

Adapted from Suhariningsih, 2004²¹

Normal dosage at this time calculation was 0.2 - 2 J per acupuncture points, and 1-4 J per trigger point. The laser doses used based on the results of the previous clinical study was described in Table 4.

To prevent ionization effect on the tissues, the applied laser dose should not be more than the ionization threshold of the body elements. It is known that emitted energy is a function of multiplication of radiated power and exposure time. Therefore, when radiation energy is emitted in a low-power laser, the dose value is determined by the length of exposure time.

Rational Basis of Laser Dose Calculation

Laser Al Ga In P which has a seven mW power was utilized for therapy in this study. If the area of the laser light output was assumed to be equal to the area of acupuncture points which were illuminated, the dose of energy could be calculated as Power (P) 7 mW = 7 x 10⁻³ joules per second. This could be interpreted as in one second; the device will emit laser light with an energy of 0.007 joules. As the desired dosage was 0.3 joules in acupuncture points, the duration of therapy as described below.²¹ Based on the data in Table 4, only a few doses were tested to select the most optimal dose. The selected doses were 0.2 Joule, 0.3 Joule, 0.4 Joule, and 0.5 Joule.

$$t = \frac{0.3 \text{ joule}}{7 \times 10^{-3} \text{ joule / sec}} = 42.86 \text{ sec} \sim 43 \text{ sec}$$

There are two basic types of lasers commonly used: first, a laser made of gas; and second, a laser made of solid material or semiconductor.^{25,26,27} The Al Ga In P laser used in this study is classified to the second type. There are few semiconductor

lasers which could be used in acupuncture therapy. First is Aluminium-Gallium-Indium-Phosphorous (Al Ga In P) with a wavelength of 650 nm, emitting red light (visible light); second is Gallium-aluminum-arsenide (Ga-Al-As) with a wavelength of 780nm-820nm-870nm; and the third is Gallium-arsenide (Ga-As) with a wavelength of 904 nm, the latter emitting infrared which is not visible. Of the three laser types, Al Ga In P is the most widely used. The main reason is due to the generated visible red light which can be more easily directed towards the acupuncture points.^{25,26,27}

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