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Laser acupuncture on ST36 promotes serum protein concentration in adolescent rats

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ABSTRACT

Background: In the last decade, laser technology has been used in medicine for diagnostic and therapeutic purposes. The low-level laser has been applied to acupoints (laser acupuncture/LA) which had potential to stimulate the expression of enzyme and hormone in various biological processes. Since the enzyme and majority of hormone is a protein or its derivatives, this study aimed to measure serum protein concentration in adolescent rats treated with laserpuncture.

Methods: Forty male three weeks old Wistar rats weighed ≥ 40 g were randomly divided into either group A or B. Each group was evenly divided into four subgroups which were a negative control and treated with LA on GV20, ST 36 or combination of GV 20 + ST 36 respectively. These acupoints were then stimulated by continuous wave cold red laser (635-680nm/5mW) generated by KX Laser GX-2000B. LA was performed at 60 seconds/day for ten days (group A) and 15 days (group B). Venous blood was collected from sinus orbitalis, and Serum protein concentration was measured using the Bradford assay. The data were analyzed using ANOVA test and p-value < 0.05 was considered as significant. Results: A higher protein concentration was observed in ST36A group (76.326 ± 15.084 mg/ml) and GV20 + ST36A group (75.044 ± 15.346 mg/ml) compared with control group A (67.338 ± 12,476 mg/ml). Meanwhile,GV20A group had lower protein level than control group at 57.838±11.042 mg/ml. By contrast, all treated rats in group B had higher protein concentrations (74.158 ± 12.754; 79.373 ± 7.265 and 77.854 ± 2.552 mg/ml respectively) compared to control group B (72.292 ± 7.987 mg/ml). However, the difference in protein concentrations in both groups did not statistically significantly ($p = 0.161$ and $p = 0.541$ respectively).

Conclusion: LA on the ST 36 acupoint has the greatest effect on increasing protein concentration in male adolescent Wistar rats although the difference was not statistically significant.

Keywords: Laser acupuncture, protein serum, adolescent, rat

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INTRODUCTION

Laser Acupuncture (LA) is defined as the stimulation of traditional acupuncture points with low-intensity and non-thermal laser irradiation. Basically, acupuncture and laser therapy are two separated therapeutic modalities, but their combination has unique synergism and potentially bolster the therapeutic effect.[1] The LA technique combines the advantages of traditional Chinese acupuncture and modern medicine.[2] Compared to traditional acupuncture, the laser has more beneficial effects such as painless, no thermal risk damage, precise doses, as well as more effective and efficient.[2,3]

Acupuncture, which is part of Traditional Chinese Medicine (TCM), has been used for more than 2,000 years. The principle of this method is inserting needles to stimulate particular acupuncture points which are divided according to its location throughout the body to obtain desired specific effects. The acupuncture can be used for prevention and treatment of several diseases. The stimulation of acupuncture points is formerly based on a classical theory which is different from Western Medicine.[4] In traditional Chinese medicine, acupuncture is related to the belief that disease is caused by disruptions in energy flow (qi) in the body. Insertion of needles will stimulate acupuncture points which release this qi and travel through qi channels, called meridians, and restoring energy flow.

Currently, acupuncture has widely practiced around the world and also has been implemented in several hospitals and other healthcare facilities. Current acupuncture is no longer based on classical theory but relied on the body's inherent regulatory system such as neuro–endocrine-immune (NEI) network which involves nervous system, endocrine system, and immune system. Currently, several studies showed that acupuncture has a specific modulatory effect on the NEI network. As the needles punctured specific points in the body, it will stimulate nerves and sends signals to the brain. Then, the brain releases neural hormones or neurotransmitters to influence targeted body systems. Other studies indicate that acupuncture decreases inflammatory markers related to the immune system.[4]

Historically, acupuncture has been developed in conjunction with technology to stimulate specific

Table 1 Concentration of Protein on Adolescent Rats

Duration of Intervention	subgroup	N	Mean	SD	Minimum	Maximum
10 day	Control	5	67.3388	12.47672	48.21	82.92
	GV20	5	57.8380	11.04277	45.80	74.16
	ST36	5	76.3272	15.08432	52.13	86.23
	GV20+ST36	5	75.0444	15.34602	49.48	86.23
15 day	Control	5	72.2924	7.98727	62.35	82.30
	GV20	5	74.1580	12.75454	61.21	90.45
	ST36	5	79.3738	7.26501	72.17	89.34
	GV20+ST36	5	77.8544	2.55223	75.17	81.10

Table 2 Statistical analysis of the differences in protein level among study groups

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Protein B	Between Groups	159.674	3	53.225	.745	.541
	Within Groups	1143.077	16	71.442		
	Total	1302.751	19			
Protein A	Between Groups	1087.486	3	362.495	1.958	.161
	Within Groups	2962.593	16	185.162		
	Total	4050.080	19			

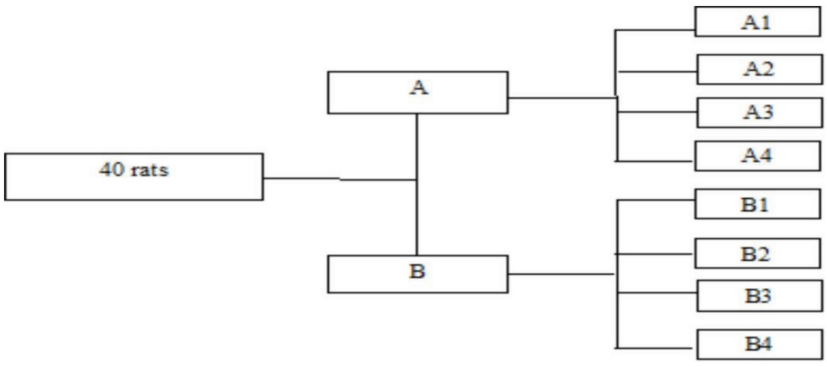

Figure 1 Study Design. 40 rats were divided into group A which treated by LA for teen days and group B which treated for 15 days. Subsequently, each group was divided into four subgroups namely A1 and B1 as a control, A2 and B2 which treated by LA on GV20, A3 and B3 which treated by LA on ST36, and A4 and B4 which treated by LA on Gv20+ST36.[11]

points by using stone, metal, needle with small electric current and, more recently, using laser technology.[2] Low-level laser, which is well known as photo-biostimulation (PBM), is a biomedical treatment that uses monochromatic light or laser irradiation to modulate biological functions.[5] PBM has been reported no serious effect and used for >50 years.[6] Nowadays, laser spectrum has been applied for a wide range of human needs over the world.1 Baxter et al.[7] concluded that LA was a more suitable form to stimulate acupuncture point than the traditional acupuncture with inserted needles, especially its application to pediatric patients. This noninvasive stimulation minimized side effects such as erythema, granuloma, bleeding and viral infections concerns (Hepatitis-B and HIV).[4]

Many studies have reported that LA application affects enzyme, hormone or specific neurotransmitter activities. Laser treatment on the skin, for example, will stimulate biological functions, such as reduction of the ROS level and LOOH formation,[8] activation of nitric oxide synthase (iNOS)9 and inhibition of inflammatory cytokines (TNF- alpha, IL-1beta, IL-6, and IL-8). Beside immunity activities, LA upregulates heat shock proteins, growth factor production (PDGF, IGF-1, NGF, and FGF-2) and protein kinase C (PKC), enhances cellular ATP levels, alters mitochondrial membrane potential due to the presence of chromophores, manipulates NF-_B activation, inhibits apoptosis and stimulates mast cell degranulation.[10] So this study aimed to investigate the effect of LA on protein serum concentration in adolescent rats.

METHODS

Animal Model

Forty-three weeks old male Wistar rats weighed more than 40 g were randomly divided into group A or B. Each group was evenly divided into four subgroups which were negative control and three

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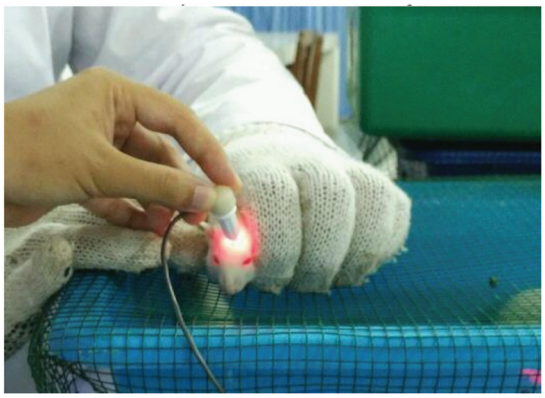

Figure 2 LA Stimulation on GV20 located on the peak of the cranium

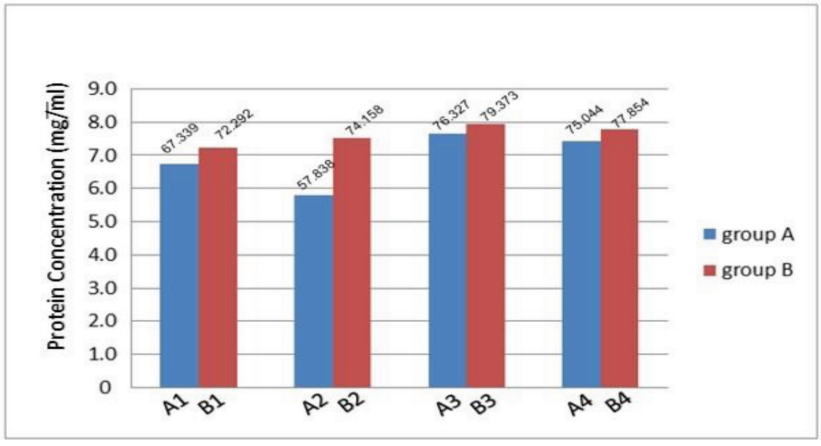

FIGURE 3 The Comparison of Mean of Serum Protein Concentration in Each Subgroup. A1. control subgroup; A2. subgroup treated with LA at GV20; A3. LA treated subgroup at ST36; A4. Subgroup treated with LA at GV20+ST36. All rats in group A received the treatment for 10days while their group B counterpart was treated for 15 days

subgroups that were stimulated by laser on GV20, ST36 or combination of GV20+ST36 respectively. These acupoints were then applied using a cold red laser (635–680nm/5mW) generated by KX Laser GX-2000B. LA was performed for 60 seconds/day for ten days (group A) and 15 days (group B) (Figure 1).

Laser-puncture Procedure

Laser Acupuncture was performed by laser stimulation using KX Laser GX-2000B (Kangxing), a semiconductor-based low-level laser therapy (LLLT) device emitting a cold red laser (635–680nm/5mW). The point ST36 and GV20 were determined before generate the laser beam. The area around the points was shaved gently using a razor, and then the laser was affixed on top of the aforementioned spot perpendicularly and turned on for 60 seconds. Control rats were treated with the inactive probe. Acupoint ST36 is located at two lower limbs, approximately 1mm lateral to the tibial tuberosity while GV20 situated at the peak of the cranium (Figure 2).

Blood Protein Level Assessment

After laser stimulation, venous blood was collected from sinus orbitalis. Subsequently, the blood sample was centrifuged at 450 rpm for 10 minutes, and the serum was isolated for protein measurement using Lowry Methods (Catalog 500-0116, Bio-rad. USA). The procedure was conducted according to the manufacturer instruction.

Statistical Analysis

Numerical data were described as means with standard deviations (SD). All data were analyzed by ANOVA using SPSS version 18.0 for Windows. P-value < 0.05 was considered as significant.

RESULTS

From the protein assessment method, the blood protein level was recorded from each group and presented as mean with SD as well as minimum and maximum value. According to the results, it appeared that ST36 group had highest protein level at both 10 and 15 days of treatment. However, stimulation both GV20 and ST36 did not give the same result although still higher than GV20 only and control group. The data of total protein serum content in all subgroups are presented in Table 1.

The comparison between groups showed that more extended stimulation at GV20 acupoint (10 days vs. 15 days) results in the highest increase in protein level compared to the other group. However, increased protein level was also observed in the control group. The comparison of the concentration of total protein serum in each subgroup is shown in figure 3.

Statistical analysis of the differences between study groups is presented in Table 2. Despite the differences observed among group A and group B, the differences were not statistically significant. Nevertheless, it should be noted that the differences between A2 and B2 subgroup are quite steep.

DISCUSSION

Determination of total proteins is widely applied for various purposes including clinical and medical researchers. In this study, we used Lowry Assay to measure protein serum concentration of adolescent rats stimulated by LA. In this method, the serum protein was reacted with, alkaline copper tartrate solution and Folin reagent. In the alkaline solution, proteins react with copper and reducing the Folin

reagent. The reduced protein species have a characteristic blue color with maximum absorbance at 750 nm.[12] Although Lowry method relatively has slow reaction rates, instability of some reagents, and nonlinearity of the standard curve, it has some benefit such as 100 times more sensitive than the biuret reaction, more straightforward method, as well as much more straightforward to adapt for small-scale analyses. [13,14] A study of the effect of LA on changes in protein serum concentration levels has never been done before. However many reports have shown that the LA positively affected body functions by stimulating hormones secretion, enhance the expression of several enzymes and other neurotransmitters.[7,8,9]

The bio-stimulating activity of low-level laser radiation of various wavelengths and energy doses is widely documented in the literature, but the mechanisms of the intracellular reactions involved still not well understood. We used semiconductor laser with emitted cold red laser (635– 680nm/5mW) continuous wave performed for 60 seconds. In a study conducted by Yeoum et al., about the effect of laser-acupuncture on IGF-1 and BMP2 in adolescent rats, increased in IGF-1 level was observed by treating the adolescent rats with a cold laser (635-680/40mW) for 60 seconds.[15] Compared with it, this study used lesser doses which may explain the non-significant increase in serum protein level. Pasternak et al. evaluated the effect of a multi-wave locked system (MLS) of two wavelengths (wavelength = 808 nm in continuous emission and 905 nm in pulsed discharge) on the human erythrocyte membrane and the secondary structure of human serum albumin (HSA).[16] Human erythrocytes membranes and HSA were irradiated with low-intensity laser light with surface energy density ranging from 0.46 to 4.9 J cm-2 and surface energy power density 195 mW cm-2 (1,000 Hz) and 230 mW cm-2 (2,000 Hz). It was reported that near-infrared laser radiation induced dose-dependent changes in erythrocyte membrane fluidity. Thus, MLS laser radiation could influence the structure and function of the human erythrocyte membrane resulting in a change in fluidity.

The proteomic response of acupuncture had been reported by Lai et al.[17] It was reported that acupuncture has potential to modulate the oxidative stress to significantly reduce blood pressure by changing of the complete protein expression profile on Spontaneously Hypertension Rats. The study of proteomic analysis in normal rat lung tissues revealed that needle stimulation in acupuncture altered proteins expression mainly through protein structural stabilization, modulating glycolysis/gluconeogenesis and response to stimuli.[18] Gao et al. showed that following electro-acupuncture (EA) procedure; there were 19 hippocampal proteins identified with more than 2-fold changes in expression, which are involved in metabolic, physiological, and cellular processes.[19] It suggested that the EA mechanism was mediated by regulation of hippocampal proteins related to amino acid metabolism and activation of the MAPK signaling pathway. On the contrary, this study assesses total protein content in the serum without specifying on certain types of protein. Therefore, we could not identify the neural, endocrine, or immune mechanism that elicited after laser treatment.

Nevertheless, changes in serum protein levels were observed in the treatment group, but these changes were inconsistent. The protein level was dropped in subgroups with ten days exposure at GV 20 while the other subgroups increased in both the 10-days and 15-days treatment. The reason for this phenomenon could be due to unstable protein concentrations and the differential effect of laser therapy between the two acupoint. GV 20 acupuncture point is located at the top of the head, so the lasers stimulated the dense bone structure and cause-focused absorption. Also, this transmission is directly penetrated to the brain. In contrast, ST 36 point which is located in the lower leg which means the laser will penetrate to loose tissues which potentially disperse the light. This acupuncture point is also located far from the brain so there could be another mechanism that reduces its effect on brain physiology.[20] However, increased serum protein was consistently reported in subgroups receiving for 15 days which could be the result of physiological adaptation. It is interesting to note that the changes did not result in a significant increase. In accordance with the current theory, acupuncture and LA will affect the body's systems to achieve balancing that can maintain and stabilize a function. Under normal circumstances, acupuncture contributes to the improvement and optimization of body's function.

CONCLUSION

To conclude, this study had examined the total protein serum of adolescent rats stimulated with LA on GV20, ST36 and both GV20 and ST36. The results indicated that LA on ST36 has highest serum concentration than the others. This study demonstrated that LA is maintaining and optimizing body system by upregulating protein serum on adolescent rats. However, the protein in this study is total protein serum, and it is not specific. Further investigation is needed to explore what protein which is involved in the NEI-network mechanism.

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