

Study to Characterize the Remote Action of Pulse Light Stimulation on SP6 In Rats: A New Physiological Phenomenon

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ABSTRACT

Background: While previous literature has documented that muscle chains or meridians (SP, LR, KI) that travel medially in the leg are all associated with parenchymatous viscera such as liver, kidney, etc., but no experimental studies have been reported. **Objectives:** To investigate the effects of pulse light stimulation (PLS) on SP6 on the deep temperature of some muscles in the medial leg and the liver and its remote action in rats. **Materials and Methods:** 80 Wistar rats were used to observe the deep temperature changes of the muscle tibial posterior (SP8) and the muscle adductor magnus (SP10) of the medial leg and the liver after the PLS (filtered LED light intensity 5 mW/cm^2 , irradiated area 0.13 cm^2 , frequency 1.25 Hz) on SP6. We also observed the effect that sciatic nerve incision or fasciotomy could have on PLS results of the experiment with the aim to explore the distal action pathway. **Results:** After PLS on SP6, the deep temperature of some muscles of the medial leg and the liver was significantly increased, and this remote action effect was significantly lower by fasciotomy lower than sciatic nerve incision. ($p < 0.05$) **Conclusion:** PLS on SP6 has a significant remote action effect on some muscles in the medial leg and liver, and its effect is much higher than that of electroacupuncture or moxibustion stimulation, and mainly is shown through the fascia.

Keywords: Pulse Light Acupuncture Stimulation; Pulse Light Stimulation (PLS), Fascia; High Thermal Line; Deep Temperature.

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INTRODUCTION

The body has information transmission pathways such as nerves, blood vessels, lymphatics, etc. Recently, the research on fascia has been conducted extensively and this has led to the recognition that fascia is also a information transmission pathway.^[7] The fascia is a membrane composed of connective tissue that surrounds all the tissue structures. And the fascia connects all the tissue structures each other.^[1,2] Thus, the connective tissue, in

combination with blood vessels, nerves, mucosa, meninges, serosa, bone, and muscles, creates multiple fascial layers, and exerts mechanical or metabolic responses to external stimuli, affecting the physiological functions of tissues.^[20] The main function of fascia is fibroblasts.^[4] Fibroblasts play an important role in mechanical force transmission. Mechanical stress in the fascia causes morphological changes in fibroblasts, and the

composition of collagen production and stroma changes, resulting in changes in the elasticity, stiffness, and thickness of the fascia.^[13,14] This action may be beneficial in protecting vessels and nerves from fascial tension, but may cause pathological changes in the proximal or distal regions.^[8-10]

Another function of the fascia is to transmit electrical information.^[18] The collagen proteins, which are the components of the fascia, have semiconductor, piezoelectric, and photoconductive properties, so that the electrical activity of the extracellular matrix changes in real time and the changes are delivered far away.^[7,15] In particular, fibroblasts have the function of receiving and transmitting biophotons^[5,16], which are influenced by several factors, such as metabolic state of the cell, the ambient temperature, and the body region.^[6,17] Thus, fascia has a large network of information in vivo, affecting the function of tissues due to its structural continuity and functional properties.^[7]

We have developed a new pulse light acupuncture stimulation method that enhances the fascial function of receiving and transmitting biophotons and, as an objective proof, has presented partial high thermal line in infrared imaging with PLS of some acupoints.^[11] However, the action pathways and characteristics of these stimulation are still unknown. Therefore, to explore the mechanism of PLS on the acupuncture point

that produces high thermal line, we investigated the remote action of PLS by using the deep temperature index.

MATERIALS AND METHODS

1. Materials

80 rats of Wistars strain weighing more than 200g were used without sex discrimination.

2. Apparatus

2.1. Pulse light acupuncture stimulator (Technical Guidelines 283-2017)

2.2. Electrostimulator (ST-3)

2.3. Deep temperature measurement device (410-2011)

2.4. Two-way Kimograph Device (BD41-04/06/NoBD 41822775, HOLLAND)

2.5. Thermostatic apparatus

3. Methods

3.1. Acupuncture stimulation method

3.1.1 Pulse light acupuncture stimulation method

After anesthesia with 1 ml/100 g of 10% urethane injected into the rat's peritoneal cavity^[23], the hair and skin of the measurement area were removed. Then, the pulse light stimulator was fixed to SP6 found by the comparative anatomy and stimulated for 20 min. The pulse light was obtained by filtering the LED light with a wavelength range of 360~760 nm through a mineral membrane which blinks at a frequency equal to the heart rate.



Figure 1. Pulse Light Extraction Procedure.

3.1.2. Moxibustion stimulation or electroacupuncture stimulation method

Moxibustion stimulation was given by burning 3 grain-sized moxa on SP6.

Electroacupuncture stimulation was done by applying a spherical impulse stimulus (0.1 ms, 2-5V) from an electrostimulator (ST-3) for 20 min to SP6 via a dipole needle probe.

3.2. Deep temperature measurement method

Deep temperature measure was done with a deep temperature meter (410-2011).^[23]

A 0.6 mm- diameter needle with a temperature sensor at its end was inserted into the tissue to be measured and the measured temperature values were recorded in real time through a two-channel recording device. The temperatures of the leg muscles and liver were measured. The deep temperature was measured in the muscle tibial posterior (SP8) and the muscle adductor magnus (SP10) of the medial leg in the study group and, this was measured in the musculus gastrocnemius (in the height of SP8) and the muscle semitendinosus (in the height of SP10) of the posterior leg in the control group.

Simultaneously, the deep temperature was measured in the parenchymal tissue of the liver. The rats with stimulation applicator and measurement needles were placed in a thermostat (temperature 28-31°C) and allowed to settle for 1 hour before the experiment.

All experiments started when the deep temperature of the measuring tissues gradually

decreased with time and remained constant within 0.1°C after anesthesia in rats.

3.3. Methods to explore the remote action pathways of PLS on the acupuncture point

The rats were dissected from sciatic nerve or after partial transection of the superior fascia of SP6, and the changes of deep temperature of the muscle tibial posterior (SP8) and liver which were stimulated by PLS on SP6 were observed.

Statistical analysis

The effect of PLS was evaluated by the difference of the maximum increase in deep temperature compared to pre-stimulation, and statistical analysis of all data was performed using SPSS 10.0 software.

RESULTS

1. Variation of deep temperature

1.1. Temperature changes in tissues of SP8 after PLS on SP6

After PLS on SP6, deep temperature changes were observed in the tissues of SP8 in the medial lower leg.

Table 1 shows that after PLS on SP6, the deep temperature of the muscle (muscle tibial posterior) in SP8 increased significantly compared to pre-stimulation, and the temperature difference between before and after stimulation increased significantly in the muscle compared to that of subcutaneous. ($p < 0.05$).

Table 1. Changes in deep temperature in the tissues of SP8 after PLS on SP6 ($\bar{x} \pm SE, ^\circ\text{C}$).

Classification	Number	Before stimulation	After stimulation	Temp. differences
subcutaneous	5	27.13 $\pm$ 0.41	27.42 $\pm$ 0.38	0.29 $\pm$ 0.01
Muscle (tibial posterior)	5	28.72 $\pm$ 0.51	29.47 $\pm$ 0.56 *	0.75 $\pm$ 0.04 Δ

* : $p < 0.05$ (comparison with before the stimulation), Δ : $p < 0.05$ (comparison with subcutaneous).

1.2. Temperature changes in leg muscles after pulse light stimulation on SP6

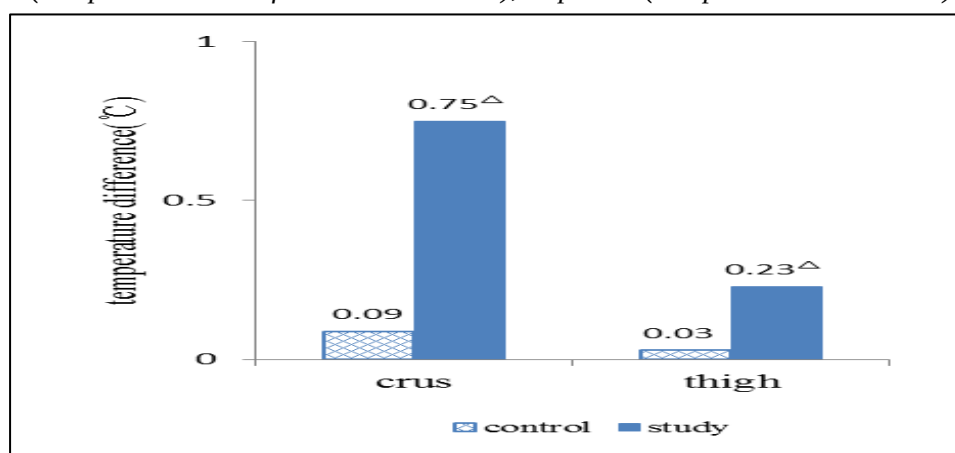
Table 2 and Fig. 2 show that after PLS on SP6, the temperature difference between before and after stimulation of the study group

(muscle tibial posterior and muscle adductor magnus) was significantly higher than that of the control group (musculus gastrocnemius and muscle semitendinosus). ($p < 0.05$).

Table 1. Deep Temperature Changes in Leg Muscles after PLS on SP6 ($\bar{x} \pm SE, ^\circ\text{C}$).

Classification			NO.	Before	After	Temp. differences
crus	Contr ol group	gastrocnemius (SP8 height)	7	27.92 $\pm$ 0.44	28.01 $\pm$ 0.46	0.09 $\pm$ 0.03
	Study group	tibialis posterior (SP8)	7	28.72 $\pm$ 0.51	29.47 $\pm$ 0.56*	0.75 $\pm$ 0.04 Δ
thigh	Contr ol group	Semitendinos us (SP10 height)	7	29.96 $\pm$ 0.37	29.99 $\pm$ 0.36	0.03 $\pm$ 0.02
	Study group	adductor magnus (SP10)	7	30.3 $\pm$ 0.28	30.53 $\pm$ 0.31*	0.23 $\pm$ 0.04 Δ

* : $p < 0.05$ (comparison with before the stimulation), Δ : $p < 0.05$ (comparison with control).



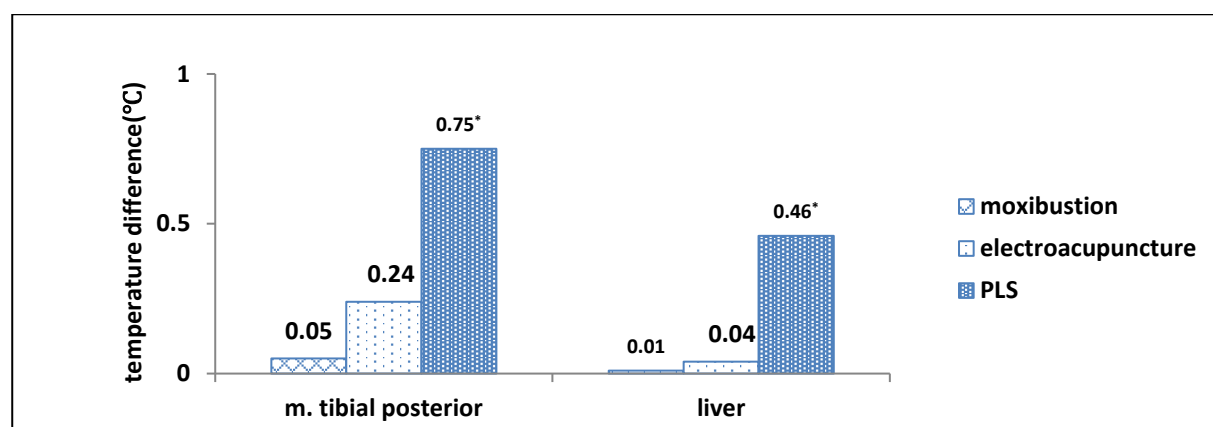
(Δ : $p < 0.05$ (comparison with control). $n = 7$)

Fig. 2. Deep temperature difference in leg muscles after PLS on SP6.

2. Changes in deep temperature following acupuncture stimulation method

After stimulation on SP6 with moxibustion, electroacupuncture and pulse light, respectively, the changes in the deep temperature of the muscle tibial posterior (SP8) and the liver were observed. As shown in Fig. 3, the deep temperature difference of the muscle tibial posterior before and after stimulation, was significantly higher in the

pulse light group as it was $0.75 \pm 0.08^\circ\text{C}$ compared that of moxibustion group ($0.05 \pm 0.03^\circ\text{C}$) and electroacupuncture group ($0.24 \pm 0.04^\circ\text{C}$), and the liver deep temperature difference was also significantly higher in the pulse light group as $0.46 \pm 0.05^\circ\text{C}$ compared that of moxibustion group ($0.01 \pm 0.005^\circ\text{C}$) and electroacupuncture group ($0.04 \pm 0.01^\circ\text{C}$). ($p < 0.05$).



(*: $p < 0.05$ (comparison with moxibustion or electroacupuncture), $n = 7$).

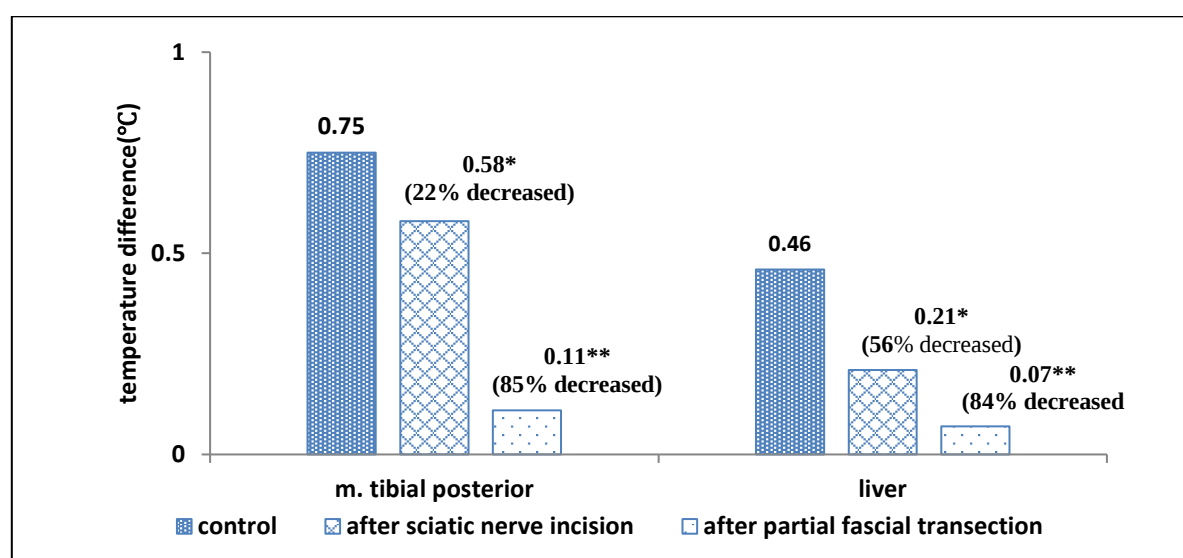
Fig. 3. Changes in the temperature difference between before and after stimulation of the muscle tibial posterior (SP8) and liver following acupuncture stimulation method.

3. The remote action Pathway of PLS on SP6

The effects of PLS on SP6 on the deep temperature of the muscle tibial posterior (SP8) and the liver were investigated after dissection of the sciatic nerve or partial transection of the superior fascia of SP6 in rats.

As shown in Fig. 4, after PLS on SP6, the muscle tibial posterior temperature difference

decreased by $22.6 \pm 0.35\%$ by sciatic nerve dissection and $85.3 \pm 0.29\%$ by fasciotomy, while the liver deep temperature difference decreased by $56.2 \pm 0.38\%$ and $84.1 \pm 0.42\%$, respectively. ($p < 0.05$). To give further objective confirmation on this result, we examined the effect of the partial transection of the fascia on the remote action of PLS on SP6 in rats with the sciatic nerve dissection.



(*: $p < 0.05$, **: $p < 0.01$ (comparison with control), $n = 7$)

Fig. 4. Effect of PLS on SP6 after sciatic nerve incision or partial fascial transection on the muscle tibial posterior (SP8) and liver deep temperature differences.

As shown in Fig 5, despite sciatic nerve dissection in the rats, deep temperature changes of the muscle tibial Posterior(SP8) and the liver continued to increase by PLS, and

when the fascia was dissected, the deep temperature decreased under PLS, and after 50 min, it returned to pre-stimulated state.

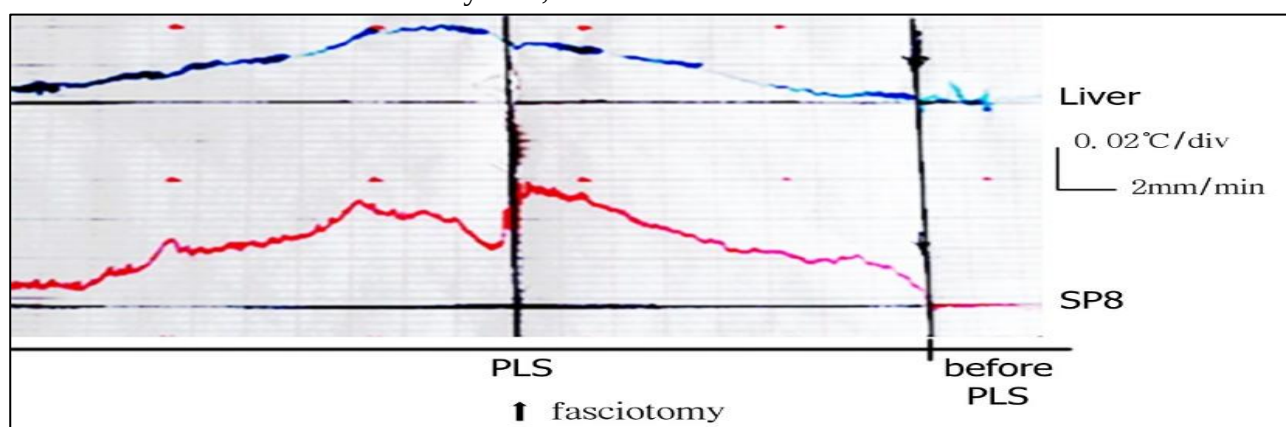


Fig. 5. Deep temperature changes of the muscle tibial Posterior (SP8) and the liver caused by PLS on SP6 before and after partial transection of fascia in sciatic nerve-amputated rats.

DISCUSSION

We developed a pulse light acupuncture stimulation method that enhances the biophotons activity of the fascia and presented high thermal lines in infrared images as stimulation to some acupuncture points.^[11] The results of this study suggest that pulse light acupuncture stimulation may have an informative effect and, furthermore, a remote action effect. We investigated the remote action of pulse light acupuncture stimulation in rats by measuring the deep temperature which sensitively reflects the tissue function.^[3,23] The literature has shown that the muscle chain(the deep front line)^[19] and the meridians (SP, LR, KI)^[21,22] that travel medially to the leg are associated with the parenchymal organs, including the liver and kidney.

We conducted experiments to evaluate the remote action of PLS on SP6 from the deep temperature of the leg muscles and the liver, based on the literature on SP6.^[21,22]

First, after PLS on SP6, the deep temperature changes were observed in the tissues of SP8 located medially to the lower leg. Results, after PLS, the deep temperature of the muscle tibial posterior of SP8 increased significantly

compared to pre-stimulation, and the temperature difference before and after stimulation increased significantly compared that of subcutaneous. ($p < 0.05$) (Table 1).

Next, we observed the effect of the deep temperature of leg muscles after PLS on SP6, where the temperature difference before and after stimulation of the muscles of the medial leg, the muscle tibial posterior and muscle adductor magnus, was significantly higher than that of the musculus gastrocnemius and muscle semitendinosus of the posterior leg muscles, respectively. ($p < 0.05$) (Table 2 and Figure 2).

The above results indicate that pulse light acupuncture stimulation affects some muscles of the leg. The effect was not on the posterior leg but on the medial leg muscles. According to the literature^[3,23], electroacupuncture on GV3 did not show significant deep temperature changes in four distal regions, including the same meridian line (GV4, GV12). These study results and our study results suggest that the remote action effects of acupoints differ according to the stimulation method. Therefore, experiments were conducted to determine whether the changes in

deep temperature of distal tissues were substantially different according to the acupuncture stimulation method (electroacupuncture, moxibustion and pulse light).

After applying electroacupuncture, moxibustion and pulse light on SP6, respectively, the deep temperature changes of the muscle tibial posterior (SP8) and the liver were observed, and the temperature difference of the muscle tibial posterior before and after stimulation was observed to be the greatest in the pulse light group, with a difference of $0.75 \pm 0.08^{\circ}\text{C}$ in the pulse light group, $0.24 \pm 0.04^{\circ}\text{C}$ in the electroacupuncture group, and $0.05 \pm 0.03^{\circ}\text{C}$ in the moxibustion group. ($p < 0.05$)

The temperature difference of the liver also significantly increased in the pulse light stimulation group as $0.46 \pm 0.05^{\circ}\text{C}$ compared to that of $0.04 \pm 0.01^{\circ}\text{C}$ in the electroacupuncture group and $0.01 \pm 0.005^{\circ}\text{C}$ in the moxibustion group. ($p < 0.05$) (Figure 3), This means that the remote action effect of PLS is much higher than that of electroacupuncture and moxibustion stimulation, and it is thought that the PLS induced the appearance of the high thermal line. Next, experiments were conducted to explore the pathways underlying the pulse light acupoint stimulation.

In general, there have been many studies on the action mechanism, the action pathways of the acupuncture stimulation, but no data on that of PLS have been published. We established the idea that PLS could be related to the fascia and conducted research to prove it.

To prove this, we examined the effect of PLS(SP6) on the deep temperatures of the muscle tibial posterior(SP8) and liver after sciatic nerve dissection or partial transection of the superior fascia of SP6, and the deep temperature difference between before and after of the stimulation of the muscle tibial posterior was reduced by $22.6 \pm 0.35\%$ by

sciatic nerve dissection and $85.3 \pm 0.29\%$ by fasciotomy, and the liver deep temperature difference was reduced by $56.2 \pm 0.38\%$ and $84.1 \pm 0.42\%$, respectively. ($p < 0.05$) (Figure 4). This suggests that the fascia plays a greater role than the nerve in the expression of the remote action of PLS on SP6. In other words, it is highly likely that the action pathways of PLS are fascia. For objective confirmation, we examined the effect of fasciotomy on the continuous PLS on SP6 in rats with sciatic nerve dissection.

The results showed that despite sciatic nerve dissection, the deep temperature of the muscle tibial posterior and the liver increased continuously by PLS, but decreased in contrast when the upper fascia of SP6 was sectioned and returned to pre-stimulation state after 50 min. (Figure 5), Thus, experiments examining the remote action of PLS on SP6 revealed that PLS has a significant remote action effect than electroacupuncture or moxibustion stimulation, and that the fascia plays a greater role than the nerve in the action pathways. Pulse light acupuncture stimulation does not cause any pain sensation unlike acupuncture. We believe that pulse light acupuncture stimulation is a new acupuncture stimulation method that further clarifies the action mechanism of the acupuncture and can be used to promote patient compliance and achieve high therapeutic efficacy.

CONCLUSION

PLS on SP6 has a significant remote action effect on some muscles in the medial leg and liver, and its effect is much higher than that of electroacupuncture or moxibustion stimulation, mainly through the fascia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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