Laserpuncture Increases Serum Concentration of Insulin-Like Growth Factor-1 in Adolescent Rats

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ABSTRACT

Objective: Previous studies have demonstrated that laserpuncture has a positive effect on longitudinal bone growth within varying parameters. The current authors hypothesized that laserpuncture affects serum concentration of insulinlike growth factor 1 (IGF-1), a growth factor that influences growth plates, and ghrelin, a growth hormone (GH) secretagogue. The goal of this research was to investigate if laserpuncture could increase serum concentrations of IGF-1 and ghrelin in adolescent rats.

Materials and Methods: This was an experimental study with a post-test only and a controlled group design. The study was performed in a laboratory on 40 male Wistar rats, age ± 3 weeks and weighing >40 g. The rats were divided randomly into 2 groups, A and B, each receiving laserpuncture for 10 days and 15 days, respectively. There were 4 subgroups in each group: control (C); GV 20; ST 36; and GV 20 + ST 36. The rats were stimulated by laserpuncture with a KX Laser GX-2000B (Kangxing), a semiconductor-based low-level laser therapy (LLLT) device emitting a cold red laser (635–680 nm/5 mW) for 60 seconds (0.3 J/cm^2). The length of each rat's lower limb was measured using a caliper; IGF-1 and ghrelin concentrations were assessed using enzyme-linked immunosorbent assays.

Results: A marked increase in IGF-1 concentration was observed in the ST 36 and GV 20 + ST 36 subgroups after 15 days, compared to the C subgroup ($P=0.001$ and $P=0.002$, respectively). The GV 20 subgroup had a lower IGF-1 concentration than the C subgroup ($P=0.06$) at both 10 and 15 days. Laserpuncture did not influence ghrelin concentrations significantly, compared to the C subgroups.

Conclusions: Laserpuncture administered to both the ST 36 and GV 20 + ST 36 subgroups with a power density of 0.3 J/cm^2 increased serum concentrations of IGF-1 in adolescent rats.

Keywords: laserpuncture, IGF-1, ghrelin, adolescent, rat

INTRODUCTION

LONGITUDINAL BONE GROWTH determines final height due to chondrogenesis at the epiphyseal plates, also known as the growth plates. This process results from

chondrocyte proliferation, hypertrophy, and extracellular matrix secretion.^{1–3} Growth hormone (GH) and insulinlike growth factor-1 (IGF-1) are the primary stimulators of longitudinal bone growth. GH stimulates synthesis and secretion of IGF-I in the liver and growth plates.^{3,4} Ghrelin, recognized

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as a “hunger” hormone, is a GH secretagogue that stimulates the GH/IGF-1 axis.⁵

Adolescence is the transitional phase from childhood through the development of secondary sexual characteristics to the achievement of final height in adulthood.^{5,6} It is a phase with rapid anatomical, physiologic, and psychologic changes. In this phase, there is an increase in early physical growth and energy requirements. Along with physical growth and puberty, many neuroendocrine factors—both systemic and local—influence this process. GH and IGF-1 are important for the acquisition of bone mass during the prepubertal phase and for maintenance of bone homeostasis throughout life. Together with physical development, these hormones will cause an increase in consumption of energy. During this phase, GH and IGF-1 drive a rising intake of food.^{6,7} IGF-1 is an anabolic hormone mainly synthesized in the liver and locally expressed in peripheral tissues. IGF-1 is under the control of GH. It seems that IGF-1 manages GH secretion through a negative feedback mechanism. GH/IGF-1 signaling is essential for healthy growth in children and for the maintenance of anabolic processes in adults.^{7–9} However, ghrelin, a 28-amino acid hormone mainly produced in the stomach, stimulates the release of GH from the anterior pituitary gland and induces a positive energy balance by increasing appetite and decreasing fat usage through GH-independent mechanisms.¹⁰

Many people try to find ways to achieve optimal height. Currently, the preferred therapy is to administer GH and IGF-1. However, this approach is difficult and expensive, and has known side-effects. Acupuncture is an alternative treatment that is safe, inexpensive, effective, and efficient. The effects of acupuncture or laserpuncture on growth factors have been reported.^{8–10}

Acupuncture, widely used in modern medicine, is a therapeutic approach that originated from Traditional Chinese Medicine. Acupuncture is useful for treating various conditions of the body and may be used for disease prevention or health promotion, or for curative properties. People believe that acupuncture can improve health and optimize physiologic functions of the body. Children, adults, and elderly people may be treated with this method.

Laserpuncture is one modality of low-level laser therapy (LLLT), a noninvasive form of phototherapy that is defined as a traditional acupuncture point stimulation with low intensity and nonthermal laser irradiation. The noninvasive and painless laser is used to provide relaxation and alleviate fears associated with the procedure in pediatric patients. Despite the use of low power, laserpuncture has the same principles of traditional acupuncture and is capable of producing the same biologic effects as acupuncture needles.^{11,12} Laserpuncture combines the advantages of traditional Chinese acupuncture with modern medicine.

Considering that acupuncture can be used to manage body systems by affecting growth factors and the hormonal

system, this study was conducted to determine the effects of laserpuncture on IGF-1 and ghrelin in adolescent rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (age ± 3 weeks) were used in this study. The rats were obtained from an animal research center with assistance from the Faculty of Pharmacy and Clinical Pharmacology at Universitas Gadjah Mada Jogjakarta. The animals were housed in standard cages at room temperature with an appropriate light cycle for day and night (i.e., the room was darkened from 17:00 to 5:00). The rats received sufficient food and drink. They were adapted to the conditions for 1 week. Then, 40 of the rats weighing >40 g were separated. By randomization, these 40 rats were placed into 2 groups, A or B. Then, each group was divided into 4 subgroups: control (C); GV 20; ST 36; and GV 20 + ST 36. The rats in group A were stimulated with the laser 10 times daily, and the rats in group B were stimulated with the laser 15 times daily. The experimental protocol was approved (number 410/V/HREC/2017) by the Medical Research Ethics Committee of the Faculty of Medicine Universitas Sebelas Maret, in Surakarta, Central Java, Indonesia.

Acupoints

GV 20, at the top of the head, and ST 36, ~ 1 mm lateral to the tibial tubercle, were used. Anatomically, GV 20 is adjacent to the brain and is associated with the frontal lobes.¹³ ST 36 was used because it promotes the release of endorphin, ghrelin, and other neurotransmitters.^{14,15}

Laserpuncture

Laserpuncture was performed by laser stimulation with a KX Laser GX-2000B (Kangxing). It is a semiconductor-based LLLT device emitting a cold red laser (635–680 nm/5 mW).

The treatment groups were GV 20, ST 36, and GV 20 + ST 36. They received laser treatments daily for 10 times (in group A) or 15 times (in group B). Subgroups GV 20 were treated with laserpuncture to GV 20. Subgroups ST 36 were stimulated on ST 36, and subgroups GV 20 + ST 36 were stimulated on both GV 20 and ST 36. Before administering the laser stimulation to each rat, the area around the point was shaved gently with a razor; then, the laser was placed perpendicularly to the skin. Laser stimulation was administered for 60 seconds (0.3 J/cm^2). For the control subgroups (C), the laser apparatus was placed on the acupuncture points without being turned on.

Limb Measurement

The length of the lower limbs of the rats were measured with a caliper. The measurement was taken from the upper part of the knee when the legs were bent to the medial malleolus of the tibia. Results were recorded in cm. The measurements were performed on day 0 of the experiment (when the animals were ± 3 weeks' old) and were repeated on day 15 (when the animals were 5 weeks' old).

Biochemical Analysis

After the treatment, a blood sample was taken from the orbital sinus of each rat. Then, the sample was centrifuged at 450 rpm for 10 minutes. The blood serum was taken and stored in a -80°C refrigerator to be used for examination of ghrelin and IGF-1 levels. The serum ghrelin level was determined by an enzyme-linked immunosorbent assay (ELISA), using a kit from Sigma (Sigma Aldrich), and the serum IGF-1 levels were determined by ELISA, using a kit from Thermo (Thermo Fisher Scientific). The researchers adhered to the procedures outlined in the package instructions.

Statistical Analysis

The data were analyzed with an analysis of variance, followed by a *post hoc* Bonferroni test, using SPSS for Windows, version 22.0. The significance level was set at $P < 0.05$.

RESULTS

Length of the Lower Limb

Before being stimulated by laserpuncture (rats age ± 3 weeks, day 0), the length of the lower limb was measured.

There was no significant difference between groups A and B. This indicated that the lengths of the limbs of all of the rats at the beginning of the experiment were homogenous. However, after laserpuncture stimulation, a marked increase in lower-limb length was observed in the ST 36 subgroup of the 15-day group, compared to the control subgroup ($P = 0.016$; Fig. 1).

Concentrations of IGF-1

The data for IGF-1 concentrations are shown in detail in Table 1. Statistically, there were significant increases in the ST 36 subgroups at 10 days and at 15 days, compared to the C subgroups. However, IGF-1 concentrations in other subgroups did not differ significantly, compared to the C subgroups (Fig. 2).

Levels of Ghrelin

The data in Table 1 show that laserpuncture influenced concentrations of ghrelin. However, statistically, this influence did not differ, compared to the C subgroups. In addition, duration of treatment did not affect these results (Fig. 3).

DISCUSSION

Length of the Lower Limb

This study showed that there was a difference in the length of the lower limb in laser-stimulated rats. The group that was stimulated by laser for 15 days had a greater length growth than the 10-day group. Meanwhile, regarding point selection, the subgroups who were stimulated at the ST 36 point had an enormous increase, followed by the subgroup that received a

FIG. 1. Comparison of lower-limb length in each subgroup. Week 3 shows the measurement of the lower limb prior to treatment (day 0). Week 5 represents measurement at the end of the experiment (day 15).

TABLE 1. MEAN $\pm$ SD OF IGF-1 AND GHRELIN IN EACH SUBGROUP

Group	Subgroup	n	IGF-1 (ng/mL)	Ghrelin (pg/mL)
A	C	5	615.30 $\pm$ 95.37	287.17 $\pm$ 118.82
	GV 20	5	494.62 $\pm$ 53.83	245.77 $\pm$ 41.48
	ST 36	5	831.37 $\pm$ 81.43	296.02 $\pm$ 122.90
	GV 20 + ST 36	5	778.10 $\pm$ 97.16	327.13 $\pm$ 24.26
B	C	5	536.05 $\pm$ 128.39	209.15 $\pm$ 61.00
	GV 20	5	460.96 $\pm$ 56.00	201.26 $\pm$ 74.27
	ST 36	5	778.67 $\pm$ 65.88	312.54 $\pm$ 159.85
	GV 20 + ST 36	5	770.49 $\pm$ 34.34	395.10 $\pm$ 140.68

Groups: A=laserpuncture at 10 days; B=laserpuncture at 15 days.

Subgroups: C=control; GV 20=laserpuncture to GV 20; ST 36=laserpuncture to ST 36; GV 20 + ST 36=laserpuncture to both GV 20 and ST 36. SD, standard deviation; IGF-1, insulinlike growth factor-1.

combination of GV 20 and ST 36 and the subgroup of GV 20 alone, compared with the control subgroups.

According to the theory, laser application will affect the local area of the acupuncture point. Laser was received as an afferent stimulus to be passed on to the spinal cord and brain as segmental and central effects. Therefore, the laser application affected the body system.^{11,12} However, statistically, there was no significant difference, except in the subgroup who received the laser treatment at ST 36 for 15 days. Perhaps this occurred because ST 36 is located in the proximal tibia; thus, it affects local conditions around the tibia directly. In addition, traditionally, ST 36 has been known to be a growth point that stimulates the growth of rat tibias. However, the mechanism of how growth occurs due to stimulation at this point is not fully known.

IGF-1

In previous studies, acupuncture and electroacupuncture (EA) at ST 36 and KI 3 increased serum IGF-1 levels and

IGF-1 renal tissue in mouse models with renal failure. However, EA increased these levels significantly more than acupuncture did.¹⁶ EA also increased IGF-1 levels considerably in osteoporotic model rats who had undergone ovariectomy.¹⁷ Meanwhile, laserpuncture at ST 36 and SP 6 increased IGF-1 levels in adolescent rats. In the current study, laser stimulation at GV 20 decreased IGF-1 levels after both 10 and 15 days of treatment. However, statistically, this result was insignificant. Meanwhile, stimulation at ST 36 and GV 20 + ST 36 increased serum IGF-1 levels. The increase occurred significantly in the 15-day group. The highest increase in IGF-1 levels was in subgroup ST 36 at 15 days, followed by subgroup GV 20 + ST 36 at 15 days. Laser administration for 10 days also increased serum IGF-1, but this was not statistically significant.

Some studies suggest that acupuncture stimulation can activate the autonomous center and the hypothalamic–pituitary–adrenal (HPA) axis. Therefore, acupuncture will maintain balance and improve the efficiency of some organism systems.^{19,20} Use of acupuncture also can influence

FIG. 2. Comparison of insulinlike growth factor-1 (IGF-1) concentration in each subgroup. Data are presented as mean $\pm$ standard deviation. * $P < 0.05$.

FIG. 3. Comparison of ghrelin concentration in each subgroup. Data are presented as mean $\pm$ standard deviation. * $P < 0.05$.

the pathway of the hypothalamic–pituitary–ovarian (HPO) axis in anovulatory patients.^{20,21} Thus, if acupuncture activates the HPA axis pathway and affects the HPO axis, acupuncture might also affect the hypothalamic–pituitary–somatomedin axis.

The current study showed that there was a decrease in IGF-1 levels in subgroups who were treated with a laser at GV 20. GV 20 is located on the top of the head. The laser, when used to stimulate this point, penetrates the thin cranium bone before finally being transmitted to the brain. It is not clear how laser stimulation of GV 20 lowers IGF-1 levels. Hamblins mentioned that laser penetration with wavelengths of 600–900 nm in the frontal area reached 21% in mice and affected brain processes directly in a way that could be useful for addressing various brain disorders.¹¹ Regarding the magnitude of the laser dose given, the Arndt-Schultz Law states that small doses stimulate, medium doses inhibit, and high doses terminate.²² The current authors suggest that, in the current study, the treatments offered were more extensive, and thus had a stunting effect.

Ghrelin

Ghrelin is a peptide hormone produced mainly by the stomach and is known as the secretagogue of GH. Ghrelin is famous for being called the “hunger hormone.” The proteins in ghrelin play a role in increasing GH secretion, food intake, and weight gain when administered both peripherally and centrally. Increased appetites in rats and humans are the result of the stimulation of the feeding-center mechanism in the hypothalamus. Ghrelin, derived from the gaster, reaches the hypothalamus through blood circulation and reaches the ventral portion of the arcuate nucleus, but the main route is through the afferent vagus nerve fibers that innervate the gastric mucosa. Another role of ghrelin is to maintain an energy balance by sending neuroendocrine signals from the gaster to the brain.^{19,20}

Several sources have reported the effects of acupuncture on ghrelin. Güçel et al. studied the effect of ghrelin on obesity, wherein an increase in ghrelin was associated with weight loss in obese women.¹⁴ Another study investigated the effects of ST 36, ST 21, and SP 6 on gastric motility and ghrelin levels in the antrum pyloricum; there was an increase in ghrelin levels.²¹ Depending on the acupoint, the data in the current study showed that there was either a decrease or an increase of ghrelin production in treatment subgroups, compared to the control groups. Group GV 20, both at 10 days and at 15 days, had a lower ghrelin concentration, compared to the C group, whereas all ST 36 and GV 20 + ST 36 subgroups had higher ghrelin concentrations. The highest increase was seen in subgroup GV 20 + ST 36 after 15 days of stimulation; there was a change in ghrelin levels, but it was not statistically significant.

The mechanism by which laserpuncture affects serum ghrelin levels is not known precisely. Some theories suggest that local stimulation is received by the receptor and transmitted by the nervous system to the visceral organs as a segmental effect and to the brain as a central effect.¹⁹ The visceral organs in this case, the gaster and intestines, have increased motility due to the autonomous system. The nucleus tractus solitarius (NTS) is in the brainstem and is recognized as the primary relay for visceral information from the cardiovascular, respiratory, and gastrointestinal (GI) systems. Studies have shown that the NTS also received somatic afferent stimuli.^{23–25} In one study, neurons from the NTS and spinal cord were labeled with neuroanatomical tracer injections via a lower-limb acupoint (ST 36) in mice.²⁴ The somatic stimulation caused by acupuncture was delivered to the NTS. The NTS is adjacent to the dorsal nucleus of the vagus motor nerve, and both form a dorsal vagal complex (DVC). The DVC integrates vago–vagal reflexes that play a significant role in the regulation of GI motor function. It has also been mentioned that acupuncture at ST 36 stimulates gastric motility through vagal efferent

nerve activity, while ST 25 inhibits gastric motility through efferent sympathetic activity.²⁶ In the current study, laser administration at GV 20 decreased ghrelin levels, although this decrease was not statistically significant.

IGF-1, Ghrelin and Length of the Lower Limb

In this study, the length of the lower limbs in the treatment subgroups increased, compared to the controls, with the highest increase in the subgroup ST 36 at 15 days of treatment. This is in contrast to the concentrations of ghrelin and IGF-1, in which laserpuncture could either decrease or increase these levels. In the subgroup GV 20, there was a decrease in the levels of ghrelin and IGF-1, whereas these levels were increased in the other subgroups. The highest increase in IGF-1 levels occurred in the subgroup ST 36, whereas the highest increase in ghrelin levels occurred in the GV 20 + ST 36 subgroups. Here, it appears that ghrelin levels did not affect the length of the lower limb significantly, compared to the effect on IGF-1 levels. This is shown by the highly elevated levels of ghrelin in the setting of lower-limb lengths that were not greatly increased. This is almost identical to the results found by Cumurdan et al., which showed that there was a negative correlation between ghrelin concentration and IGF-1 in short-statured adolescents; IGF-1 was shown to affect bone mass, while ghrelin did not affect it.²⁷ Bone development is more dependent on IGF-1 levels than ghrelin.

CONCLUSIONS

Laserpuncture at ST 36 significantly increased lower limb lengths and IGF-1 concentrations in adolescent rats.

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The experimental protocol for this research was approved (number 410/V/HREC/2017) by the Medical Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret, in Surakarta, Central Java, Indonesia.

AUTHOR DISCLOSURE STATEMENT

No competing financial interest exist.

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