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Lumbar Sympathetic Ganglion Block Facilitates Wound Healing in a Rat Ischemic Hindquarter Model

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Background: Lumbar sympathetic nerve block (LSNB) improves blood flow in the lower limbs and relieves pain involving the sympathetic afferents. This study examines the use of LSNB, but there are no reports of its use for the purpose of wound healing. Therefore, the authors planned the following study.
Methods: An ischemic limb ulcer was created on both lower limbs using a rat model (N = 18). The rats were divided into three groups, namely, A, B, and C. Group A received LSNB on one side (N = 6). Group B was sprayed with basic fibroblast growth factor preparation (trafermin/fiblast) on one side (N = 6). Group C was used as a control (N = 6). Lower limb temperature and the ulcer area were measured over time in each group. Furthermore, the correlation between the ulcer temperature and the ulcer area reduction rate was analyzed.
Results: Group A had higher skin temperature on the LSNB-treated side than on the nontreated side ($P = 0.0022 < 0.05$). Regarding the correlation between the average temperature and the ulcer area reduction rate, the correlation coefficient was as high as 0.691 in group A.
Conclusions: In the LSNB group, the skin temperature increased and the ulcer area decreased significantly. Conventionally, LSNB has been used for pain relief purposes, although the authors consider that it will be useful in the treatment of ischemic ulcers and that it is a potential treatment option for future chronic limb ischemia/chronic limb-threatening ischemia cases. *(Plast Reconstr Surg Glob Open 2023; 11:e5010; doi: 10.1097/GOX.0000000000005010; Published online 24 May 2023.)*

INTRODUCTION

A globally aging population and the accompanying increase in lifestyle-related diseases, such as hypertension, diabetes, and dyslipidemia, will lead to increasing cases of chronic limb ischemia (CLI) and chronic limb-threatening ischemia (CLTI). Existing treatments, such as revascularization and surgery, are well established for CLTI; however, revascularization is not successful in all cases, and moreover, some patients are ineligible (including cases defined as "no-option CLI"). Therefore, alternative treatment options are urgently required.

Lumbar sympathetic nerve block (LSNB) involves the sympathetic afferents and provides pain relief while improving blood flow to the lower limbs. LSNB is used for lower limb obstructive diseases and diabetic lower limb ischemia. However, applying LSNB for wound healing has not been previously reported. LSNB improves blood flow, increases the skin temperature, and increases the skin perfusion pressure.[1,2] Therefore, the authors postulated that its mechanism of action may improve wound healing, particularly in cases of ischemic ulcers. The authors created a rat model of ischemic limb ulcer to investigate the hypothesis that LSNB would promote ulcer healing by improving blood flow to the limb.

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METHODS

Male Wistar rats (Nippon SLC Co., Ltd., Shizuoka Prefecture, Japan) were used. All rats were 16 weeks of age at the start of the experiment. This study was approved by the relevant ethics review board (reference number: 577-2-42, 2021-028). Ischemic limb ulcer models were created in both lower limbs of 18 rats. The rats were divided into three groups: A, B, and C. Group A underwent LSNB on one side (N = 6). Group B was sprayed with basic fibroblast growth factor (bFGF) preparation (trafermin/fiblast) once a day on one side (N = 6). Group C was used as the control (N = 6) (Fig. 1). The skin temperature and ulcer area of each group were measured.

The animal models were created according to the following steps.

Takeaways

Question: Does lumbar sympathetic nerve block (LSNB) promote ulcer healing by improving blood flow to the limb?

Findings: We used a rat model of ischemic limb ulcer. LSNB-treated group had higher skin temperature than on the non-treated side ($P = 0.0022 < 0.05$), and the ulcer area decreased significantly ($P = 0.0022 < 0.05$).

Meaning: Conventionally, LSNB has been used for pain relief purposes, although we consider that it will be useful in the treatment of ischemic ulcers and that it is a potential treatment option for future CLI/CLTI cases.

Creation of Ischemic Limb Ulcer Models

The models were created with the rats under inhalation anesthesia using isoflurane. The femoral artery was ligated just below the inguinal ligament using a 4-0 silk thread. Additionally, as previously reported,[3] the site was ligated with a 4-0 silk thread after the branch of the lateral femoral circumflex artery and before the branch of the popliteal artery to interrupt blood flow from the collateral circulation through the thigh muscle group as much as possible (Fig. 2).

Creation of Ulcer Models

A plastic plate with a 12×8 mm hole was placed on the dorsal aspect of the rat's foot, and the hole was filled with crystal violet. The same part was excised in all layers of the skin. At this time, the extensor tendon tissue remained as reported in the previous literature[4] (Fig. 3).

LSNB

The procedure was performed with the rats under inhalation anesthesia using isoflurane. The L3 and 4 vertebral bodies were confirmed under ultrasound, and the sympathetic trunk present in the compartment comprising the psoas major fascia and the posterior lobe of the renal fascia was identified. Furthermore, 0.1 mL of absolute ethanol was injected in the compartment around the sympathetic trunk.

LSNB improves blood flow to the lower limbs and relieves pain involving the sympathetic afferents in lower limb obstructive diseases and diabetic lower limb ischemia. The procedure was performed with the rats awake. One spray of bFGF preparation (trafermin/fiblast) was applied to the ulcer (6 μg as the main component). The ulcer creation date was set as day 0, and progress was observed once per day until day 21, when the observation was completed.

Outcomes

Measurement of Skin Temperature

Skin temperature in each lower limb was measured using a smart phone-mounted infrared camera (FLIR ONE Pro: Teledyne FLIR: Phoenix, AZ).[5,6] Measurements were taken at 7, 14, and 21 days after LSNB. The measurement was performed with the rats under anesthesia with isoflurane.

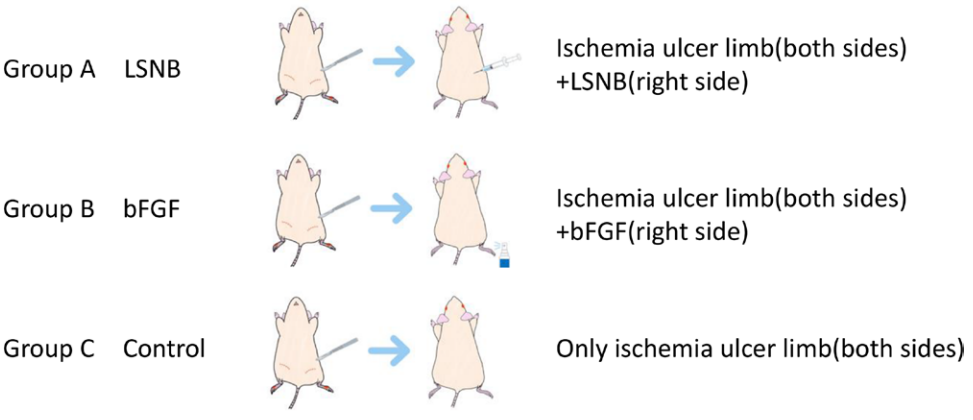

Fig. 1. The animals were divided into three groups (A, B, and C), with six animals in each group, for a total of 18 animals. In all groups, the femoral arteries on both sides were ligated. Group A: LSNB was performed on one side only. Group B: The bFGF preparation was sprayed on one side only. Group C: control group.

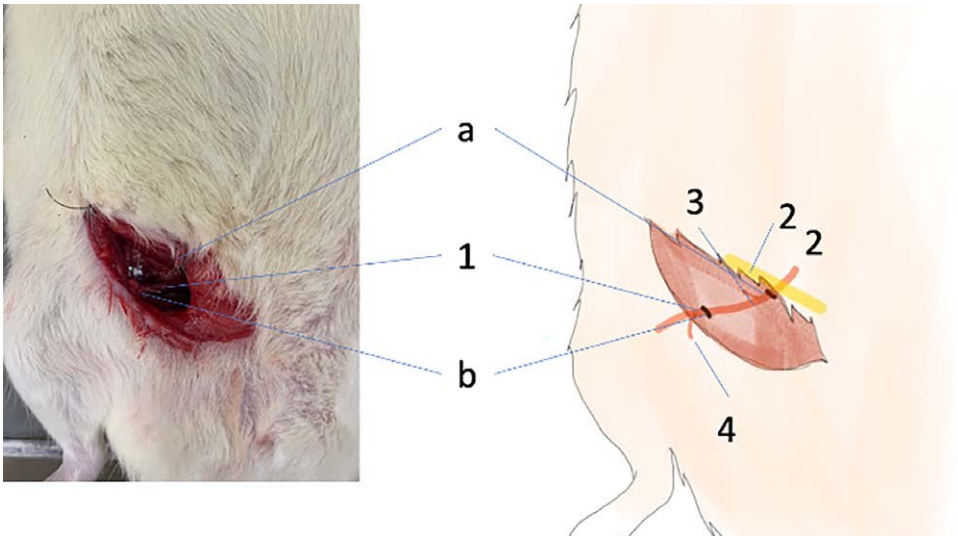

Fig. 2. Creation of an ischemic limb model. Ligate the femoral artery (1) in two places: a, directly below the inguinal ligament (2) and b, lateral femoral circumflex artery (3) postbranch/popliteal artery (4) prebranch. Rats have many collateral vessels, and to reduce the inflow from the quadriceps and hamstrings to the femoral artery as much as possible, femoral artery ligation was performed at two locations: directly below the inguinal ligament and above the popliteal artery branch.

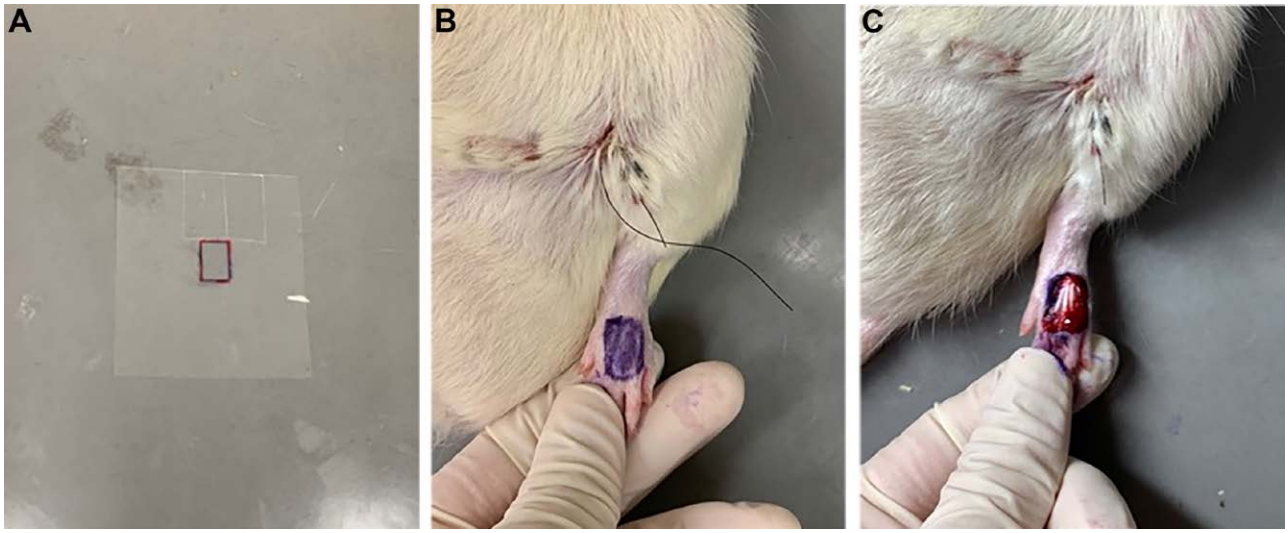

Fig. 3. Creation of an ulcer model. A, Create a plastic plate with a hole of 12×8 mm. B, Place the plate on the back of the rat's foot and fill the hole with crystal violet. C, Excise the same part with a scalpel, removing all layers of the skin and exposing the epithelium of the extensor tendons.

Measurement of the Ulcer Area

The authors used free open source software (ImajeJ: National Institutes of Health: Bethesda, MD). The area was measured by marking the original ulcer and the remaining ulcer on the same photograph. The measurement was performed on day 14 after ulcer creation, and the residual ulcer area percentage (%) was calculated using the following formula:

Residual ulcer area (%) = (remaining ulcer area/original ulcer area) × 100.

Correlation between the Increase in Skin Temperature and the Ulcer Area Reduction

On the blocked side of group A, the correlation between the ulcer temperature and the ulcer area reduction was analyzed on day 14 after the start of the experiment. Similarly, the correlation between temperature and the ulcer reduction on the side to which bFGF was applied and on the side without bFGF was analyzed. The correlation between temperature and the ulcer reduction was also analyzed by combining the control sides of groups A, B, and C.

Table 1. Temperature Change. Top: Temperature Change in Group A. Middle: Temperature Change in Group B. Bottom: Temperature Change in Group C

Rat Number	Ulcer Side	First Week	Second Week	Third Week
1	right	32.8	33.4	34
	left	30.3	31.6	32.3
2	right	30.9	30.9	33.8
	left	28.7	30.7	32.4
3	right	31.5	31.8	34
	left	28.8	27.5	31.1
4	right	31.2	32	31.5
	left	29.6	31.5	30.8
5	right	29.5	31.5	30.6
	left	28.8	30.5	29.9
6	right	31	32.4	32.4
	left	30.5	31.7	31.2
Rat Number		First Week	Second Week	Third Week
7	right	31.5	28.1	28.3
	left	31	29.4	28.5
8	right	31.6	30.5	29.5
	left	30.9	28.6	30.8
9	right	29.2	30.1	29.6
	left	30.3	29.3	31
10	right	30	29.6	30.6
	left	30.6	31.4	31.2
11	right	28.7	27.9	28.1
	left	28.1	28.1	28.1
12	right	31.5	28.6	27.2
	left	31	29.1	27.4
Rat Number		First Week	Second Week	Third Week
13	right	28.2	26.5	28.6
	left	29.6	28.5	29
14	right	28.7	26.5	29.3
	left	28.4	25.4	29.1
15	right	30.5	29	27.4
	left	29.8	28.5	27.5
16	right	26.6	29.2	28.4
	left	27.7	29.4	28.8
17	right	30.3	24.3	25.7
	left	29.9	25.7	25.5
18	right	30.6	28	26.1
	left	30.2	27.5	26.3

Analysis

The measurements were categorized into two groups: the left foot (control group) and the right foot (group A, LSNB; group B, bFGF; and group C, ulcer formation only). Statistical analysis was performed using the Student *t* test. A *P* value less than 0.5 was considered statistically significant.

RESULTS

Temperature Change

In group A, all rats had a higher skin temperature on the LSNB-treated side than on the nontreated side throughout the experiment (Table 1). The average temperature difference was 1.700°C 1 week after LSNB, 1.417°C after 2 weeks, and 1.433°C after 3 weeks. These differences were significantly greater on the LSNB side for all measurement points ($P < 0.05$). [**See tables, Supplemental Digital Content 1**, which displays (a) the temperature change in group A, (b) temperature change in group B, and (c) temperature change in group C, **http://links.lww.com/PRSGO/C563**.] No significant temperature difference was observed in group B following the application of bFGF. No significant temperature difference was seen in the control group (Table 1; **see tables, Supplemental Digital Content 1**, **http://links.lww.com/PRSGO/C563**.).

Residual Ulcer Area

In group A, the average residual ulcer area was 29.454% on the LSNB side and 46.607% on the control side, demonstrating a significant reduction in the ulcer area on the LSNB side ($P = 0.0022 < 0.05$). In group B, the average residual ulcer area was 14.464% on the bFGF side and 22.548% on the control side. The ulcer

area reduction on the right side sprayed with bFGF was smaller, although this difference was not significant $P > 0.05$). The average residual ulcer area rate in group C was 37.107% on the right side and 36.080% on the left side, showing no difference between the left and right sides ($P = 0.919 > 0.05$) (Table 2). [**See tables, Supplemental Digital Content 2**, which displays (a) changes in the ulcer area in group A, (b) changes in the ulcer area in group B, and (c) changes in ulcer area in group C, **http://links.lww.com/PRSGO/C564**.]

Correlation between Average Temperature and Ulcer Area Reduction

A relationship was examined between the average temperature and ulcer area reduction in the second week in the non-LSNB and non-bFGF groups (N = 24) between the LSNB side (N = 6) in group A and the bFGF side (N = 6) in group B. The average temperature and the ulcer area reduction in each group were plotted on a Cartesian plane, and the correlation between these values and the linear regression equation was calculated. A correlation coefficient of 0.691 was observed in the LSNB group, whereas a weak correlation was observed in the other groups. (**See tables, Supplemental Digital Content 3**, which displays (a) the plot of temperature and ulcer area reduction rate at 2 weeks in group A (LSNB performed foot n = 6), (b) plot of temperature and ulcer area reduction rate at 2 weeks in group B (bFGF-treated foot n = 6), and (c) plot of temperature and ulcer area reduction rate at 2 weeks in the ischemia-only group C (control foot n = 24), **http://links.lww.com/PRSGO/C565**.)

DISCUSSION

LSNB reversibly or irreversibly destroys the function of the sympathetic ganglia in the lumbar region, improves blood flow in the lower limbs, prevents sweating, and relieves pain involving the sympathetic afferents.[7,8] LSNB is indicated for a number of medical conditions including peripheral vascular disorders, such as arteriosclerosis obliterans, Buerger disease, and Raynaud syndrome; pain associated with the sympathetic nervous system such as in complex regional pain syndrome; and nonneuronal lower back pain (nonmedullary lower back pain) such as in spinal stenosis.[9] The authors observed improvement in refractory ulcers following LSNB in clinical practice. However, the effect of LSNB alone on reducing the ulcer area has not been previously reported. LSNB is often performed by anesthesiologists, who have fewer opportunities to examine wounds. Furthermore, plastic surgeons, who have many opportunities to examine the wounds, have limited knowledge of LSNB and minimal experience in its clinical application. These aspects hinder the selection of LSNB as a treatment option. The gap in knowledge between clinicians with different fields of expertise may be the reason why the use of LSNB for wound healing has not yet progressed substantially.

Wounds generally heal through an inflammatory mechanism, a proliferative phase, and a mature phase. The inflammatory phase comprises a series of inflammatory reactions from hemostasis to wound cleansing after injury. In patients with ischemic ulcers, decreased blood flow prolongs the inflammatory phase due to tissue hypoxia, malnutrition-induced infections, and persistent edema. During the proliferative phase, fibroblasts produce collagen fibers, develop capillaries, and form granulation tissue, which are inhibited in patients with decreased blood flow. In patients with diabetes and chronic renal failure requiring hemodialysis, desensitization due to neuropathy, decreased immune function, and severe calcification of the arteries also occur in addition to blood circulation disorders.[10,11] The coexistence of these conditions may impede treatment. LSNB increases the skin temperature and skin perfusion pressure due to its vasodilatory effect, and in principle, has the potential to shorten the inflammatory phase and promote the proliferative phase by providing abundant blood flow to the tissue, thereby promoting wound healing. There are reports indicating that sympathetic ganglion block increased blood flow in animal models.

1. Brachial artery blood flow increased after stellate ganglion block (SGB) in dogs. SGB increased brachial artery blood flow significantly in dogs.[12]
2. Blood flow of the common carotid artery, lingual mucosa, mandibular bone marrow, and masseter muscle increased in rabbit SGB on the side of the block.[13]

There is also a report that cervical sympathetic ganglion block increased blood flow, promoted repair of the intestinal epithelium, and improved the barrier function of the intestinal wall in the intestinal mucosa following radiation and burn injury in rats.[14] However, there are no reports that lumbar sympathetic ganglion block improved blood flow in the leg and accelerated the healing of ulcers.

In this study, an increase in skin temperature was observed throughout the experimental period, and the ulcer area was significantly reduced in the LSNB group. The authors believe that this occurred because LSNB relaxed the vascular smooth muscle of the lower limbs, dilated the blood vessels, and improved blood flow. When the correlation between skin temperature and ulcer area reduction was examined, there was a strong correlation in the LSNB group and no correlation in the bFGF and control groups. Although an association was observed in the LSNB group, the skin temperature alone did not correlate with the ulcer area; thus, it is possible that various factors other than skin temperature are involved in the reduction of the ulcer area. For example, persistent pain causes sympathetic nerves to become hypertonic and causes vasoconstriction[1]; therefore, it is possible that LSNB-induced pain contributed to ulcer healing. It has been suggested that ulcer healing-promoting factors such as the expression of CD34-positive cells, as described in the following section regarding hyperthermia, may be involved.[15] BFGF specifically binds to fibroblast growth factor receptors present in vascular endothelial cells and fibroblasts. Wound healing is promoted by angiogenic and granulation-promoting effects, although no significant difference in the reduction in ulcer size was observed in this study. In one rat

Table 2. Ulcer Area Reduction Rate in Each Group. Top: Changes in the Ulcer Area in Group A. Middle: Changes in the Ulcer Area in Group B. Bottom: Changes in the Ulcer Area in Group C

Rat Number	Ulcer Area	Right	Left
1	Original ulcer area	48,248	41,274
	Remaining ulcer area	5185	6716
	The ulcer area residual rate	0.10746559	0.16271745
2	Original ulcer area	61,047	61,906
	Remaining ulcer area	22,794	31,108
	The ulcer area residual rate	0.37338444	0.5025038
3	Original ulcer area	71,216	89,734
	Remaining ulcer area	12,924	40,334
	The ulcer area residual rate	0.18147607	0.44948403
4	Original ulcer area	89,242	76,848
	Remaining ulcer area	27,726	39,750
	The ulcer area residual rate	0.31068331	0.51725484
5	Original ulcer area	1,23,698	75,982
	Remaining ulcer area	53,184	45,480
	The ulcer area residual rate	0.42995036	0.59856282
6	Original ulcer area	8243	8252
	Remaining ulcer area	3003	4670
	The ulcer area residual rate	0.36430911	0.56592341
Rat Number		Right	Left
7	Original ulcer area	8762	8087
	Remaining ulcer area	850	1051
	The ulcer area residual rate	0.09700982	0.12996167
8	Original ulcer area	10,798	10,833
	Remaining ulcer area	888	1713
	The ulcer area residual rate	0.08223745	0.15812794
9	Original ulcer area	7477	9684
	Remaining ulcer area	723	3702
	The ulcer area residual rate	0.09669654	0.38228005
10	Original ulcer area	11,126	9454
	Remaining ulcer area	3162	1310
	The ulcer area residual rate	0.28419917	0.13856569
11	Original ulcer area	9152	9042
	Remaining ulcer area	1143	3798
	The ulcer area residual rate	0.12489073	0.42003981
12	Original ulcer area	9088	9285
	Remaining ulcer area	1661	1150
	The ulcer area residual rate	0.18276849	0.12385568
Rat Number		Right	Left
13	Original ulcer area	6633	11,353
	Remaining ulcer area	1149	927
	The ulcer area residual rate	0.17322479	0.08165243
14	Original ulcer area	8429	11,568
	Remaining ulcer area	1563	6461
	The ulcer area residual rate	0.18543125	0.55852351
15	Original ulcer area	3854	4805
	Remaining ulcer area	1568	2329
	The ulcer area residual rate	0.40685003	0.48470343
16	Original ulcer area	8592	10,595
	Remaining ulcer area	1912	2200
	The ulcer area residual rate	0.22253259	0.20764512
17	Original ulcer area	8951	7222
	Remaining ulcer area	3802	2686
	the ulcer area residual rate	0.42475701	0.37191914
18	Original ulcer area	6502	9617
	Remaining ulcer area	5290	4427
	The ulcer area residual rate	0.81359582	0.46033066

in the non-bFGF subgroup of group B, shrinkage of the ulcer was observed. However, the rats did not have a neck collar; thus, in the bFGF group, it cannot be excluded that the rats licked the wound, although no licking behavior was observed, and that the bFGF preparation affected the results on the nonspray side through the bedding.

CLI accounts for 20%–30% of atherosclerosis obliterans cases and is characterized by resting pain and tissue defects in the lower extremities lasting for at least 2 weeks. The disease can rapidly become life-threatening if infection progresses and induces systemic inflammatory response syndrome.[16,17] According to the TASC II guidelines, the 1-year mortality rate in patients with CLI is approximately 20%, and they have an extremely poor prognosis.[18] The first treatment is to induce revascularization as soon as possible. Reconstruction includes endovascular treatment using a catheter comprising a balloon or stent treatment and bypass surgery using an autologous blood vessel or an artificial blood vessel using an autologous vein. However, patients with no-option CLI who have unsuccessful revascularization may require conservative treatment such as drug and ulcer treatments; therefore, a new treatment strategy is urgently required. Several treatments have been attempted for patients with no-option CLI and patients with CLTI as follows. Infrared therapy has been reported to be effective for pressure ulcers and skin ulcers,[19,20] and a study suggests that thermal therapy using a dry sauna at 60˚ improves ischemic ulcers by improving the ankle-brachial pressure index and blood flow. In addition to dilating peripheral blood vessels, hyperthermia has also been shown to induce CD34-positive cells, which are vascular endothelial progenitor cells, in the peripheral blood.[11,21] Accordingly, this could be a potential ulcer treatment strategy. LSNB exerts a peripheral vasodilatory effect that is not mediated by heat, although it is possible that substances with ulcer-improving or epithelialization-promoting effects are released due to an increase in local blood flow and an increase in the temperature of the affected limb. Spinal cord stimulation (SCS) is recommended for patients with CLTI for whom revascularization is not indicated. This treatment relieves intractable pain and improves blood flow by passing a weak electric current through the spinal cord. Electrodes placed in the epidural space retrogradely stimulate sensory fibers to release vasodilators to the periphery, which relaxes vascular smooth muscle, dilates peripheral blood vessels, and improves microcirculation.[22] Furthermore, SesCS suppresses sympathetic vasoconstriction by inhibiting nicotinic transmission in the sympathetic nerve at the ganglion level.[23]

There is a possibility that the patient may not accept the idea of an indwelling foreign body and that there is a risk of infection due to the indwelling electrodes. However, it has been reported that the 1-year limb salvage rate after SCS is as high as 83%, and the National Institute for Health and Care Excellence in the United Kingdom recognizes SCS as a cost-effective treatment. However, inclusion and exclusion criteria apply when considering SCS in patients, and the indication and timing of treatment are important. LDL adsorption-type blood purification (LDL apheresis) uses an LDL-adsorbed blood purifier that adsorbs and removes LDL-C in patients with arteriosclerosis obliterans and is indicated for intractable foot ulcers. Improvement in the ankle-brachial index and improvement in the walking distance in patients with a limp have been reported.[24] The main disadvantages of this treatment are that it is expensive, requires blood access, and is time-consuming. Hyperbaric oxygen therapy involves placing the patient in a 100% oxygen high-pressure environment, which may be used for ischemic ulcers.[25] In patients with diabetic foot ulcers and peripheral arterial occlusive disease, hyperbaric oxygen therapy has been reported to reduce amputation rates.[26] However, only a limited number of facilities are equipped with hyperbaric chambers.

LSNB presents a low risk of infection as it does not involve a foreign body and is more versatile than SCS with no adaptation restrictions on prognosis or the ulcer diameter. The authors suggest that LSNB should be considered as an option for managing intractable ulcers and used in conjunction with other treatments. Regarding future research, our research group aims to collect cases and report on the clinical application of this treatment and on decisions about the indications for treatment.

In this research, the authors demonstrated the usefulness of LSNB for wound healing. However, several limitations should be noted. This study reproduced the ischemic lower limb model; however, the cell function was normal because healthy rats were used. It is necessary to verify whether LSNB is effective in rats with diabetes and severe ischemic lower limbs in which stem cells of the vascular system are abnormal. Also, while LSNB may be used in the clinical application for chronic ulcers, we used LSNB for acute ulcers in this study, implying that the origin of wound may technically differ. We would like to identify the true clinical indications for LSNB and continue our research to determine whether a similar facilitation of wound healing is observed in clinical practice in the presence of many confounders.

LSNB was performed on ischemic limb ulcer rat models to examine its role in promoting ulcer healing. LSNB significantly increased the skin temperature and significantly reduced the ulcer area. Conventionally, LSNB has been used for pain relief purposes, although the authors believe that it can be effectively applied in the treatment of ischemic ulcers and that it is a potential treatment option for future CLI/CLTI cases.

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DISCLOSURE

The authors have no financial interest to declare in relation to the content of this article.

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