Master of Engineering

Impact of treadmill exercise after lymph node dissection followed by radiotherapy in a rodent model

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Department of Biomedical Science

Sang Ah Kim

Impact of treadmill exercise after lymph node dissection followed by radiotherapy in a rodent model

Supervisor: Jae Yong Jeon, MD, PhD

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Sang Ah Kim

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This certifies that the dissertation of Sang Ah Kim is approved.

Committee Chair: Won Kim, MD, PhD

Committee Member: Jae Yong Jeon, MD, PhD

Committee Member: Eun Jae Ko, MD, PhD

Department of Biomedical Science

Ulsan, Korea

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어느 덧 2 년의 시간이 흘러 석사과정의 매듭을 짓다 보니 이 과정 속에서 스쳐간 인연들과 감사한 분들이 떠오릅니다.

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I am writing to express my heartfelt gratitude for the support and encouragement you have provided me throughout our journey together. Nessa, thank you for being there for me with your warm encouragement and love. The memories we created together here will forever hold a special place in my heart. Ben, I am grateful for your constant belief in my abilities. Your words of encouragement, "You can do it," have pushed me to persevere in every endeavor. I cannot thank you enough for this.

그리고 당연한 하루는 없다는 것을 일깨워준 사랑하는 내 친구 김유정, 타지 생활에 즐거움을 더해주고 긴 시간 지켜온 우정으로 곁에서 힘이 되어줘서 고맙습니다. 멀리서도 아낌없는 응원과 격려를 보내준 친구들 모두 고맙고 사랑합니다.

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덧붙여 본 실험과정에서 희생된 동물들에 애도를 표하며, 감사함을 가슴 깊이 새기고 그 숭고함을 잊지 않도록 하겠습니다.

ABSTRACT

Background and Purpose Lymphedema is a chronic condition caused by radiotherapy and surgery, and exercise has been reported to have a positive effect on this condition. However, exercise-induced changes in the lymphatic system and skin after lymph node dissection and radiotherapy are not well established. Therefore, the aim of this study was to examine alterations in lymphatic drainage pathway, skin thickness, and the extent of fibrosis in rats with lymph node dissection and radiotherapy after treadmill exercise.

Materials and Methods 12 Sprague-Dawley rats were divided into exercise and control groups of 6 rats each, and lymphedema was induced by popliteal and inguinal lymph node dissection followed by irradiation. The exercise group underwent treadmill exercise for 30 minutes per day, five days a week for four weeks. The hind limbs were imaged using indocyanine green (ICG) lymphography every other week during the exercise period to visualize lymphatic function, while ankle thickness was measured weekly to assess changes in the extent of swelling. Histopathological analysis was conducted at the end of the exercise period to examine dermal thickness, lymphatic vessel density, and the degree of fibrosis.

Results Exercise after surgery and irradiation resulted in improved lymphatic fluid retention. At week 3, the ICG lymphography showed that the group that performed exercise had a higher occurrence of linear and splash patterns. In addition, Ankle thickness was consistently lower in the exercise group, but there was exclusively significant difference between exercise and control groups at week 4 ($p= .016$). The group that underwent exercise showed a thinner epidermis and dermis compared to the control group ($p = .041$ and .002 each), as well as a lower percentage of collagen area ($p = .002$) and a higher lymph vessel density ($p = .002$).

Conclusion The results show that exercise may have a positive effect on the pathology of the lymphatic system after treatment that may induce lymphedema, including surgery and radiotherapy.

Keywords lymph node dissection; radiotherapy; lymphedema; treadmill exercise; ICG lymphography; rat model

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List of Abbreviations

CDT	Complex decongestive therapy
ICG	Indocyanine green
H&E	Hematoxylin and eosin
MT	Masson's trichrome
IHC	Immunohistochemistry
HPF	High-power field

INTRODUCTION

Lymphedema is a chronic disease characterized by the abnormal buildup of fluid rich in proteins within the interstitial space resulting in increased limb volume, caused by lymphatic dysfunction associated with lymph pumping, lymph fluid flow, and valve function [1, 2]. Patients with gynecological cancer may develop secondary lymphedema as a result of radiotherapy and surgical procedures such as lymph node dissection. About forty percentage of patients with gynecology cancer is suffering from lymphedema worldwide [2, 3]. It is important to emphasize the significance of addressing lymphedema treatments, as it goes beyond the mere extent of abnormal interstitial fluid accumulation. Over time, it can progress to fibrosis and lead to compromised physical function and reduced quality of life, often accompanied by recurrent infections [4].

In clinical practice, complex decongestive therapy (CDT) is widely employed as a conservative treatment approach for lymphedema. This comprehensive therapy includes various components such as manual lymphatic drainage massage, exercise, skin care, and the use of compression garments. These interventions are implemented in combination to effectively manage and alleviate lymphedema symptoms[4, 5]. CDT is a widely adopted strategy aimed at alleviating symptoms, enhancing quality of life, and managing chronic lymphedema. This approach not only minimizes the burden on patients but also plays a vital role in the treatment of lymphedema.

Traditionally, active movement or exercise was not recommended as a treatment for lymphedema. However, over the past decade, there has been an increasing acknowledgment of the importance that exercise can help alleviate the condition of lymphedema. The previous studies have reported that therapeutic exercise following axillary lymph node dissection can help prevent the onset of lymphedema associated with breast cancer [5-7].

Indocyanine green (ICG) lymphography can be used to investigate lymphatic function in vivo. ICG lymphography staging represents the severity of dermal backflow according to changes in patients with lymphedema [8]. Previous studies have utilized rats in order to observe lymphatic drainage patterns via ICG lymphography [9, 10]. They have reported that in a hind limb non-operative rat model, a linear signal was observed, while in the operative hind limb after removal of popliteal and inguinal lymph node, dermal backflow patterns were shown.

Nevertheless, the effect of exercise following surgery and radiotherapy on alterations in skin and lymphatic function, such as drainage patterns, is not yet fully understood. Therefore, Animal studies can provide valuable insights into understanding the influence of exercise on lymphedema resulting from lymph node dissection and radiotherapy. This study aimed to assess the impact of exercise on lymphedematous hind limbs in a rat model, which were induced by lymph node dissection and irradiation. The evaluation involved analyzing the lymphatic drainage patterns using the ICG lymphography and measuring the extent of edema, fibrosis, and lymphatic vessels density.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of the Asan Medical Center, in Seoul, South Korea approved all animal procedures. We used 15 female Sprague Dawley (SD) rats (JA BIO Corporation, Republic of Korea) aged 8 weeks and weighing between 250-300g. The rats were housed in a controlled environment with regulated temperature and lighting. The 12 rats were divided into two groups, with 6 in the exercise group and 6 in the control group. Additionally, out of the 15 rats, 3 were selected for a subgroup analysis to compare the effects of popliteal lymph node removal alone versus popliteal and inguinal lymph node removal together. The experimental timelines illustrating all the experimental procedures have been presented in Figure 1.

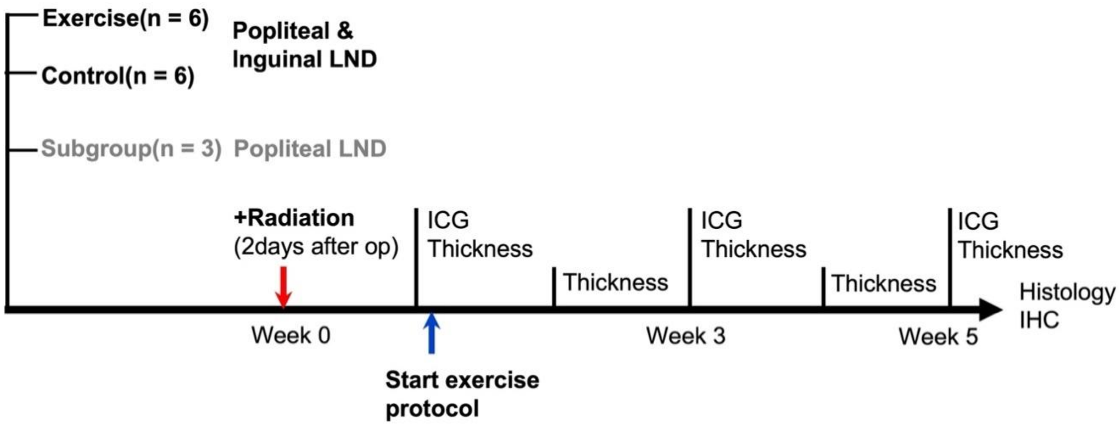

Figure 1. Schematic illustration of the experimental timelines. Abbreviations: LND, lymph node dissection; op, operation; ICG, indocyanine green lymphography; IHC, immunohistochemistry staining.

Experimental procedure

On the first day of the experiment, to induce a hind limb lymphedema rat model, the rats were anesthetized by inhaling 4% isoflurane and given an injection of a 5:1 mixture of Zoletil (Tiletamine/Zolazepam, Virbac, France) and Rumpum (Bayer Korea, Republic of Korea) at a dose of 10 ml per kg of the rat's body weight. The inguinal and popliteal lymph nodes on the left side of the rats were then surgically removed. Before the surgical procedure, we prepared the rats by shaving them with a hair clipper and using depilatory creams. To locate the inguinal and popliteal lymph nodes, we injected 30 μl of a solution containing Evans blue dye (30 mg/ml) into the hind paw and the left base of the tail intradermally, respectively (see Figure 2A). Following this, we created a circumferential through the skin to reach the subcutaneous tissue in the left groin area, and successfully identified the inguinal and popliteal lymph nodes that had been marked with the dye. (Figure 2B and C). The incision was created at a position above the lymph nodes (see Figure 2D), followed by the surgical removal of the lymph nodes and the adjacent adipose tissue. Rats in the subgroup underwent a surgical procedure to remove only the popliteal lymph node. The skin that was cut was treated with heat to stop bleeding, a process called cauterization, using a

Bovie instrument (Bovie® Medical Corporation, item no. 18010-00, USA). To ensure that the chronic lymphedema models were stable, we used 4-0 nylon to suture the skin edges to the fascia, leaving a 5mm gap between them, which we called the gap suture [11]. Two days after this procedure, we administered a single 20Gy dose of radiation using an X-ray machine (X-Rad320, Precision, CT, USA) [12], while the rest of the area was covered with a plate except for the left groin area. The follow-up period for this study was 4 weeks.

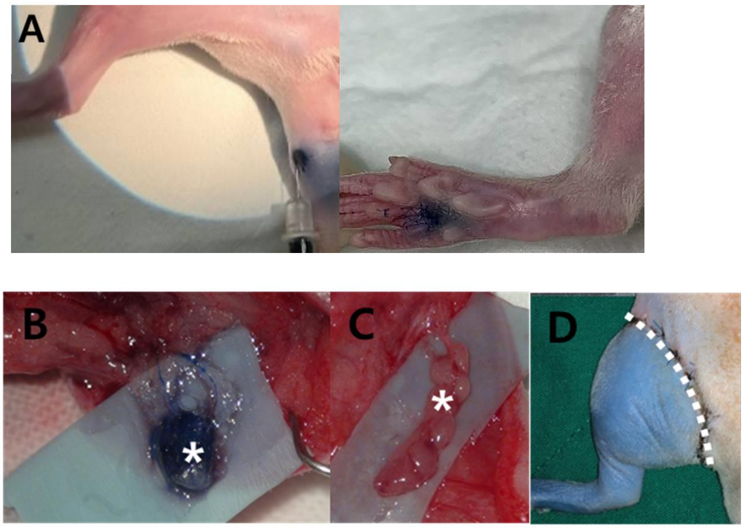

Figure 2. Photographs depicting the steps involved in the experimental procedure. **A,** injections are administered at the base of the tail (left) and hind footpad (right). **B and C,** the locations of the lymph nodes (indicated by an asterisk) are shown: the popliteal lymph node in B and the inguinal lymph node in C. **D**, the dotted line represents the incision line above the placement of the inguinal lymph node.

A protocol for treadmill exercise

The rats in the exercise group were given exercise training starting one week after the operation. They were familiarized with the treadmill (LT320, No. 99-0070, HYB®, China) by progressively the speed over a duration of five days, starting from 5m/min and reaching 12m/min during the first week [13, 14]. The exercise regime involved 30 minutes of exercise per day, five days a week, for a period of four weeks. Treadmill exercise was set at moderate intensity and speed at 10-12 m/min[13, 14]. The rats in the control group and subgroup did not receive any treatment after the surgery.

Thickness difference between both ankles

To evaluate the degree of edema, we measured the thickness of the ankle (10 mm above the heel) using Image J software weekly (see Figure 3). Prior to measurement, rats were shaved using clippers and hair removal cream. Rats in the exercise group were photographed by the same experimenter lying on the same dish under 4% isoflurane inhalation before exercise. Control group and subgroup of rats underwent the same procedures as rats in exercise group except that they did not participate in treadmill exercise. If the difference in volume between the initial measurements was greater than 5%, it was considered to be edema caused by lymphedema [14].

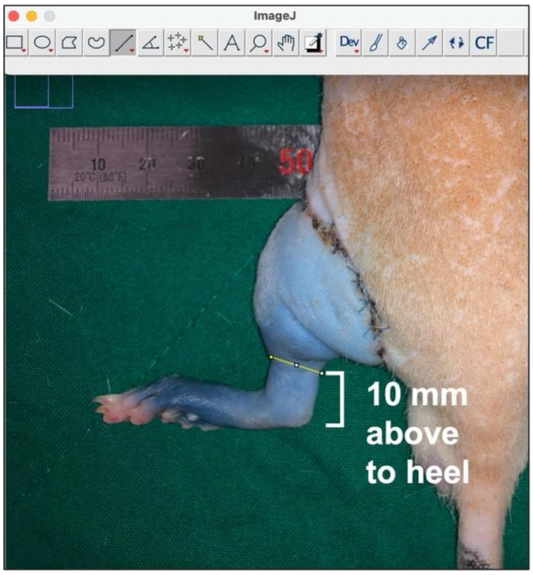

Figure 3. Measurement region of ankle thickness with imageJ software.

ICG lymphography staging in lymphedematous limb

We used a special camera equipped with a bandpass filter (LST1-01G01-FRD1-00, Opulent Americas, NC, USA; FF01-832/27-50-D; Semrock, NY, USA; USA) and a 730nm high-power LED to capture an image of the lymphatic pattern (as shown in Figure 4A) [14]. Under inhalational anesthesia, 30 μl of a mixture of 2.5 mg/ml bovine serum albumin and 1 mg/ml ICG was injected intradermally into dorsal side of the hind paw of the rat [14]. We massaged the injection site and hind limb to facilitate the absorption of the ICG solution into the lymphatic vessels, after which we captured images of the operated hind limb. We collected ICG images of the ventral proximal part of the hind limb, then classified them into five patterns and scored them according to severity, as described in a previous study [8, 10] (see Figure 4B). This process was repeated every other week, starting on the first day of the exercise program.

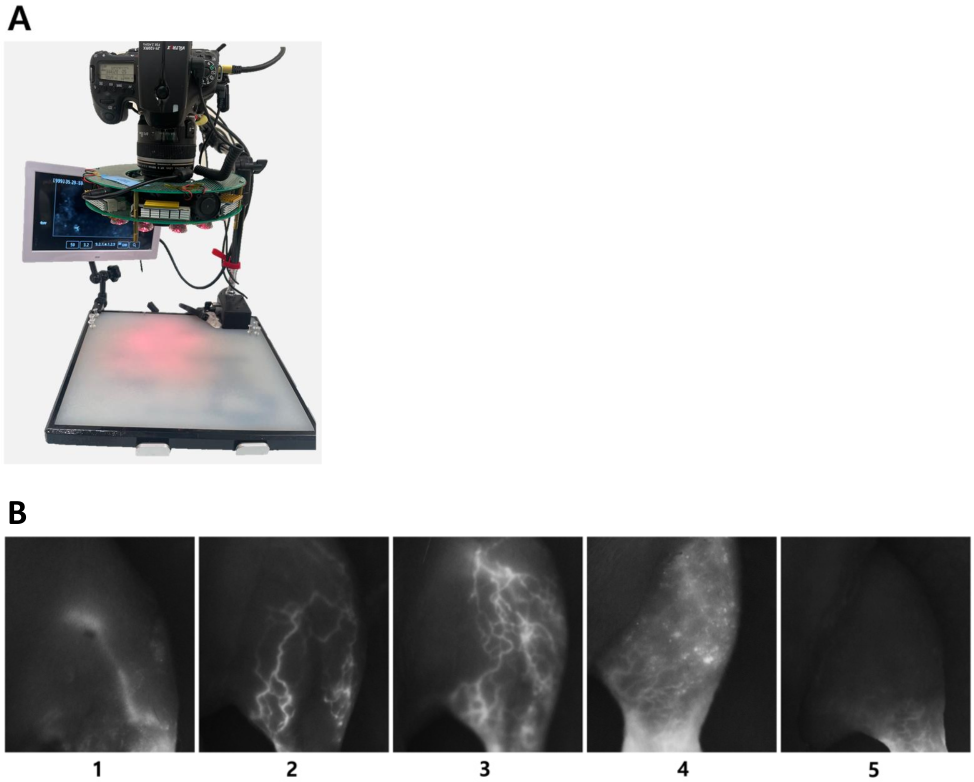

Figure 4. Visualization and categorization of the lymphatic pathway patterns. A, A specialized camera to visualize lymphatic patterns. **B,** The images obtained were classified based on the appearance of the lymphatic vessels as follows [14]: i) score 1 (linear), which had clear and distinct lines; ii) score 2 (splash), where the linear vessels were intermixed with other lymphatic vessels that looked winding; iii) score 3

(stardust), which showed shining dots like stars along with dimly lit lymphatic vessels; iv) score 4 (diffuse), which had a blurry appearance without clear lymphatic vessels; and v) score 5 (none), where no pattern was visible.

Histopathological examination

After the experimental period, rats were euthanized in a carbon dioxide cage, and both hindlimbs were immediately harvested. The samples from the hind limb were fixed by immersing them in a solution of 4% buffered formalin at room temperature for a period of 14 hours and then subjected to decalcification. Afterwards, we conducted Hematoxylin and eosin (H&E) staining as well as Masson's trichrome (MT) staining to investigate the thickness of the skin and collagen area fraction (%), respectively. After that, 48 hind limb samples were embedded in paraffin and then sliced into sections that were 5-µm-thick [14]. Finally, each specimen was stained with H&E and MT, and microscopic analysis was conducted. With the aid of an Olympus microscope (BX40, Olympus, Japan) and cellSens imaging software (Standard, Olympus), four fields were randomly selected from each specimen for analysis by a blinded examiner. The data was subsequently computed in accordance with a previously described protocol [15]. To account for age-appropriate changes in growth and animal size, each data point was normalized using the following formula: (operated hind limb - non-operated hind limb) / (operated hind limb + non-operated hind limb).

Analysis of Immunohistochemistry

To visualize lymphatic vessels, twelve hind limb specimens underwent Immunohistochemistry (IHC) staining using a mouse monoclonal antibody D2-40 (ACR266B, Biocare Medical, CA, USA) that binds to podoplanin present in lymphatic endothelium at a 1:100 dilution. Four fields were randomly selected from each specimen for analysis by a blinded investigator with an Olympus microscope (BX40, Olympus, Japan) and cellSens imaging software (Standard, Olympus). Subsequent data were calculated according to previously described protocols [15]. To account for age-appropriate changes in growth and animal size, each data point was normalized using the following formula: (operated hind limb - non-operated hind limb) / (operated hind limb + non-operated hind limb).

Statistical analysis

The data is shown as mean ± standard deviation (SD) values and analyzed with GraphPad Prism 9 (GraphPad Software, Inc., San Diego, CA, USA). To compare two groups, the Mann-Whitney test was used. To make comparisons across different time points, one-way analysis of variance (ANOVA) was used, followed by the post-hoc Tukey's test. The Wilcoxon matched-pairs signed rank test was utilized to assess changes in the indocyanine green (ICG) patterns over time and compare between the two groups, with statistical significance determined at $p < .05$.

RESULTS

Post-operative exercise improves swelling in lymphedematous hind limb of rat model

Weekly measurements of ankle thickness were conducted, and the percentage of difference in ankle thickness between both sides was determined 1 week after surgery (see Table 1). On day 0, there was not a significant difference in the thickness of the ankles among the two groups. However, as shown in Figure 5 and Table 1, the difference peaked at week 1, just before starting exercise (exercise group: 1.829 ± 0.655 mm vs. control group: 2.130 ± 0.464 mm, mean ± SD). The average ratio of ankle thickness difference between both sides in the exercise and control groups was 11.7% and 11.1%, respectively. With time, both groups experienced a reduction in lymphedema. Starting from week 2, the values began to decrease, and by week 4, rats in exercise group had a significantly smaller difference in ankle thickness compared to control group ($p = 0.016$, Mann-Whitney U test). In Table 1, it was found that the degree of swelling in exercise group significantly decreased at weeks 3, 4, and 5 compared to week 1 ($p = 0.018$, 0.030, and 0.030 each, Turkey post hoc test). In the subgroup, the average of the ratio of the difference in ankle thickness between both sides was about 1.0%. Additionally, there was no significant difference in the time course of ankle thickness due to less swelling over the five weeks in rats with popliteal lymph node dissection compared to the other groups (At week 1, subgroup: 0.29 ± 0.229 mm, mean ± SD). Based on these findings, it is suggested that the removal of the inguinal lymph node should be considered when establishing a lymphedema model.

Table 1. The difference in the weekly measurements of ankle thickness.

	Exercise	Control	Subgroup	*P-value*
Week 0	0.177 ± 0.171	0.034 ± 0.161	0.095 ± 0.0221	0.485
Week 1	1.829 ± 0.655	2.130 ± 0.464	0.290 ± 0.229	0.15
Week 2	1.155 ± 0.708	1.617 ± 1.118	0.216 ± 0.140	0.631
Week 3	0.325 ± 0.363††	1.008 ± 0.510†	0.176 ± 0.102	0.055
Week 4	0.312 ± 0.232††	0.950 ± 0.460†	0.160 ± 0.188	0.016*
Week 5	0.319 ± 0.242†	0.519 ± 0.430†	0.046 ± 0.049	0.423
P -value	<.001[a]	0.002[a]	0.251	

[a] Significantly different among weeks (P<.01, One-way ANOVA)
†P<.05, ††P<.01, Significantly different between week1 and each week (Turkey post hoc test)
*Significantly different between exercise and control groups for each week (P<.05, Mann-Whitney U test)

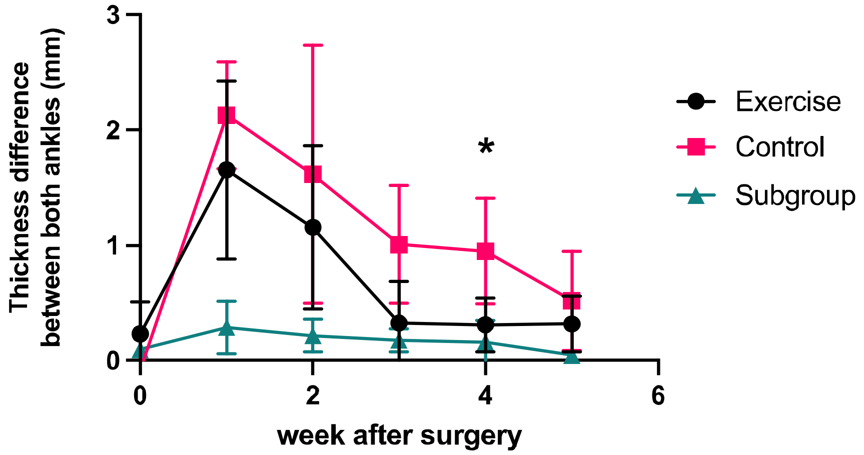

Figure 5. Comparison between exercise, control, and subgroup of thickness difference between both ankles measured with image J. In the 4th week, there was a significant difference in thickness of both hind limb between the control and exercise groups (n = 6 per group; * $p < .05$, Mann-Whitney U test). The subgroup (triangle) represents a group of rats from which only popliteal lymph node has been removed (n = 3). The subgroup consistently exhibited lower edema in the hind limb compared to the other two groups throughout all time periods.

Changes in the severity of lymphedema via ICG lymphography staging

In Table 2 and Figure 6, the scores of both groups were almost the same during week 1 of the study. The exercise group had a score of 4.2 ± 1.1, while the control group had a score of 4.0 ± 1.0 (mean ± SD) at week 1. According to Table 2, during week 3, rats in the exercise group showed more linear and splash patterns compared to those in the control group. Fifty percent of the rats in the exercise group showed these patterns, while only 17% of the rats in the control group exhibited them. Furthermore, the severity of the patterns in the exercise group was significantly improved compared to the control group (p = 0.094 vs. 0.375 each, according to the Wilcoxon signed-rank test). The number of patterns for each group was also indicated. Additionally, in week 5 of the study, new collateral pathways were found in the groin region above the incision line, extending towards the axillary region. One rat demonstrated this phenomenon, as shown in Figure 7A-C.

Table 2. Classification of the ICG pattern (%) and assessment of severity scores in both the exercise and control groups.

ICG lymphography, n(%)	Week 1	Week 3	Week 5
Exercise Group (n=6)			
Linear	0(0)	2(33.3)	3(50)
Splash with linear	1(16.7)	1(16.7)	1(16.7)
Stardust	0(0)	2(33.3)	1(16.7)
Diffuse	2(33.3)	1(16.7)	0(0)
None	3(50)	0(0)	1(16.7)
Control Group (n=6)			
Linear	0(0)	0(0)	2(33.3)
Splash with linear	1(16.7)	1(16.7)	3(50)
Stardust	0(0)	3(50)	1(16.7)
Diffuse	3(50)	2(33.3)	0(0)

None	2(33.3)	0(0)	0(0)

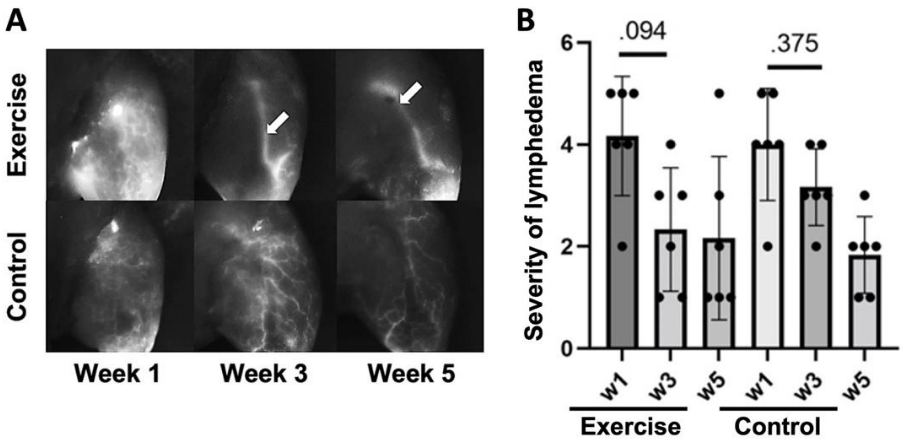

Figure 6. Comparing ICG lymphography patterns on ventroproximal between the exercise group and the control group. A, Representative images of ICG lymphography in hind limbs taken at weeks 1, 3, and 5, with linear signals indicated by arrows**. B,** The time course of ICG pattern was examined in both groups.

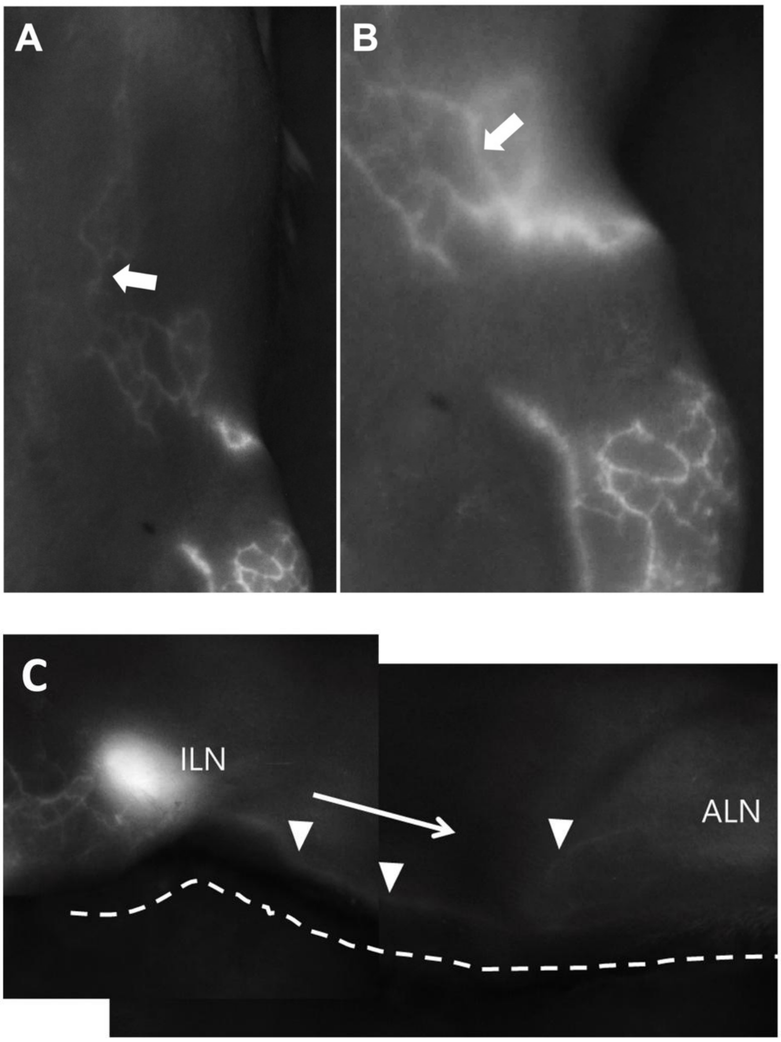

Figure 7. Representative images of ICG lymphography for a rat in exercise group (n=1, A and B) and for rats in the the group with only ventral popliteal lymph node removal (n=3, C) at 4 weeks. The images were taken on the ventral side of the rat's abdominal area (A) and thigh area (B). The arrows indicate the newly observed pathways extended from the groin region beyond the incision line towards the axillary

region. In panel C, a representative ICG image of rats with the removal of popliteal lymph node alone on the ventral side are shown. The arrowheads indicate preexisting lymph vessels connected with inguinal lymph node toward axillary lymph node along the lateral intermodal vessels. Abbreviations: ILN, Inguinal lymph node; ALN, Axillary lymph node.

Decrease in thickness of epidermis and dermis

In Figure 8A-B, specimens that were stained with H&E and belonged to the control group exhibited thickening of the epidermis when compared to the exercise group. Specifically, the epidermal thickness was measured to be 0.22 ± 0.07 μm in the control group and 0.12 ± 0.06 μm in the exercise group.

Increase in the number of lymphatic vessels

The density of lymphatic vessels at the site of lymph node dissection was significantly greater in the exercise group compared to the control group. The number of lymphatic vessels was measured to be 4.7 ± 0.6 /HPF in the exercise group and 3.0 ± 0.4 /HPF in the control group (see Figure 8C-D).

Reduction of the extent of fibrosis

The exercise group showed a significantly smaller collagen area compared to the control group. The percentage of collagen area was measured to be 0.02 ± 0.01% in the exercise group and 0.09 ± 0.03% in the control group (see Figure 8E-F).

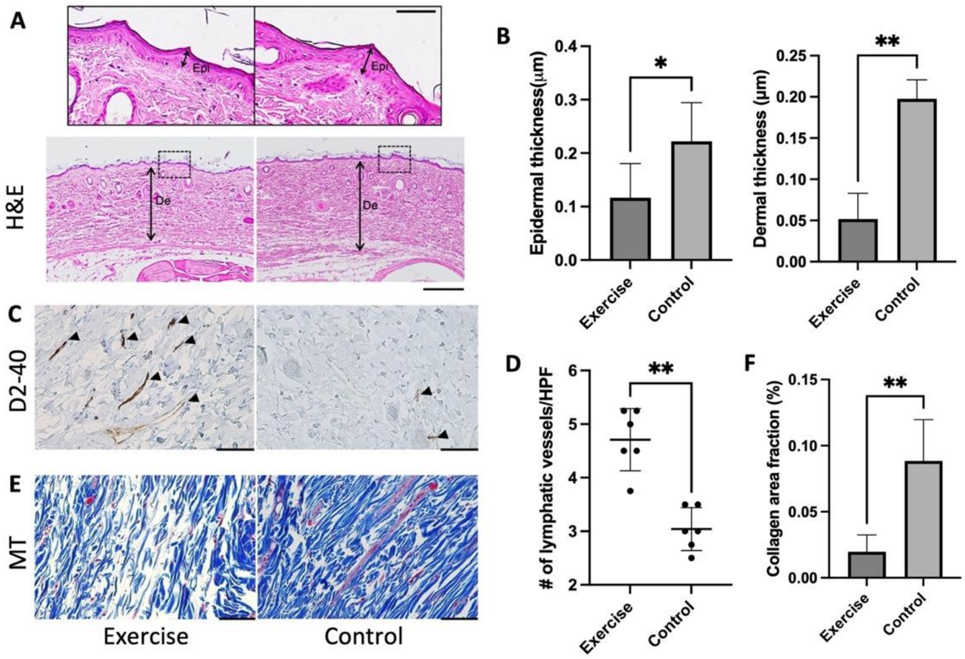

Figure 8. Comparison of changes in skin thickness, the lymphatic vessel density, and the extent of fibrosis after exercise period between the exercise group and the control group. A and B, Representative images of the epidermis and dermis stained with H&E. The rats in the control group had significantly thicker skin compared to the exercise group (n = 6 per group, Mann-Whitney U test: p = .041 and .002, epidermis and dermis, respectively). **C and D**, After exercise, the lymphatic vessel density per high-power field (HPF) significantly increased, and positive podoplanin lymphatic vessels were indicated by arrowheads (n = 6 per group, Mann-Whitney U test: p

= .002). **E and F**, The percentage of the collagen area fraction was significantly higher in the control group than in the exercise group (n = 6 per group, Mann-Whitney U test: p = .002). The scale bar for A was set at 50 μm and 200 μm each, while C and E were set at 50 μm. The original magnification for A was ×200 and ×40, respectively, while C and E were ×400. Abbreviations: Ep, epidermis; De, Dermis; H&E, hematoxylin and eosin; MT, Masson Trichome. The significance levels were indicated by asterisks (*<.05, **<.01).

DISCUSSION

To the best of our knowledge, this study is the first experimental investigation of the impact of exercise on lymphedema resulting from lymph node dissection and surgery in a rat model. We showed that exercise significantly reduced swelling in the affected hind limb of rats with lymphedema caused by lymph node dissection and radiotherapy. Additionally, this study suggests that exercise may aid in the recovery of the lymphatic system based on improvements in ICG lymphography patterns and the number of lymphatic vessels in the affected limb.

Rat model has been used to elucidate the mechanism of treatments on lymphedema since it is repeatable and can be examined by histopathology. Previous studies have explored the creation of hind limb lymphedema rat models while considering the associated morbidity and mortality [3, 11, 16]. Using insights from these studies, we employed a combination of a 20 Gy radiation dose, removal of lymph nodes, and gap suture to create our experimental model. Huang CW et al. were able to establish rat models of hind limb lymphedema by excising both the popliteal and inguinal lymph nodes in addition to making a circumferential incision to obstruct collateral lymphatic vessels [17]. In a subgroup analysis of our study, we observed that the difference in thickness between both sides was significantly lower at week 1 when maximum lymphedema typically occurs (as indicated by the triangle shape in Figure 5). These findings further support the notion that the removal of both the popliteal and inguinal lymph nodes, along with a circumferential incision, should be performed to establish a relatively chronic model.

Several experimental studies performed treadmill exercise to explicate the effect of physical activity and aerobic exercise for several diseases such as stroke, neurodegenerative disease, and obesity [18-20]. The studies highlighted that exercise should be employed in addition to other treatments. In the present study, we observed that treadmill exercise facilitated reduction of swelling in lymphedematous hind limb of rats. The extent of swelling was sharply decreased in both groups at week 2, and then it was gradually decreased during the exercise period. Our findings suggest that aerobic exercise in the form of a treadmill may help relieve lymphedema. This could be attributed to the combined effects of muscle pumping and improved blood and lymphatic circulation. The flow of lymphatic fluid is regulated by both extrinsic and intrinsic forces, including the action of skeletal muscles and smooth muscle contraction within the lymphatic vessels [20]. Moreover, the lymphatic valve is a crucial structure that facilitates the unidirectional flow of lymphatic fluid, which prevents the accumulation of fluid in downstream lymphatic vessels. To maintain this function, a continuous and unidirectional flow of lymphatic fluid is necessary [22, 23], which can be propelled by the action of skeletal muscle pumping. Based on this understanding, it can be postulated that exercise may be beneficial in preventing and treating lymphedema.

In this study, the ICG lymphography staging was employed to examine the alterations in lymphatic patterns. The percentage of dermal backflow patterns in the exercise group rats was observed to be lower than that in the control group rats at week 3, suggesting that rats subjected to the exercise protocol may have exhibited earlier recovery from lymphedema compared to the control group. Furthermore, the severity scores showed similar results in both groups by the final week of the exercise period. This could be attributed to the comparatively faster recovery rate observed in rats, which may differ from the recovery rate observed in humans.

Previous studies have shown that, in the hind limb, collateral pathways typically form after disruption of the existing lymphatic pathway and often extend towards the inguinal and axillary lymph nodes as a compensatory mechanism [11, 24, 25]. Consistent with these findings, we observed lymphatic vessels extending towards the axillary lymph nodes from the inguinal lymph nodes (n=1) beyond the incision line following exercise, even in cases where the lymph nodes had been removed due to the operation. Previous studies have reported that the majority of rats with removal of the popliteal lymph nodes exhibit collateral pathways towards the axillary lymph nodes after the operation, and these pathways usually run along the lateral side of the internodal blood vessels [11, 25, 26]. However, in our study, We showed that in rats where both the popliteal and inguinal lymph nodes were removed, new collateral pathways were observed on the ventro-cranial side in the lateral region (Figure 5 D). This may explain the relatively chronic hind limb lymphedema model induced in this study, as it is plausible that the lymphatic fluid requires a longer period to traverse this route. Our findings suggest that removal of both the popliteal and inguinal lymph nodes is necessary to induce a chronic hind limb lymphedema model.

We examined images of H&E staining under a microscope to observe the epidermis of rats from the control group and found it to be thicker than those from the exercise group, possibly due to accumulated interstitial fluid and fibrosis of the cutaneous tissue. Our findings on the collagen fraction area (%) is consistent with this speculation, as they indicate that rats who underwent exercise had better fibrosis compared to those who did not. Previous study compared the epidermal thickness of rats with lymphedema to a control group and found that the lymphedema group had significantly thicker epidermis. This thickening may be caused by the accumulation of lymphatic fluid flowing back into the epidermis and turning into adipose tissue, a process known as dermal backflow.

Our findings suggest that severe lymphedema can lead to epidermal thickening and fibrosis, but exercise may help alleviate these symptoms. We support this claim with evidence of higher lymphatic vessel density in rats of the exercise group.

The current study has some limitations. Firstly, although rat lymphedema models have a lymphatic structure that is similar to humans, they cannot completely replicate the human body because rats usually recuperate more quickly from lymphatic system blockage. However, animal studies can provide valuable insights into the progression and treatment of chronic diseases that last for a long time but can be observed in a relatively short period. Our experimental study lasted for at least 4 weeks, as suggested by previous studies on treadmill exercise, to determine the impact of physical activity. Our findings on the chronic model up to 5 weeks provide useful information for future studies on secondary lymphedema. Secondly, our model was unable to confirm the preventive effects of postoperative exercise before lymphedema occurs. Lastly, due to the limited sample sizes, it is difficult to achieve statistical significance and apply these findings to humans. Therefore, we recommend that future researchers conduct studies with larger sample sizes.

CONCLUSIONS

This study provided evidence that treadmill exercise could alleviate lymphedema conditions, including fibrosis and the degree of swelling, and facilitate the restoration of lymphatic drainage pathways in rats subjected to popliteal and inguinal lymph node dissection along with radiation therapy. Therefore, we suggest that aerobic exercise may help alleviate lymphedema caused by lymph node dissection and radiotherapy.

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Korean Abstract

설치류 모델에서 림프절 절제 및 방사선 요법 후 트레드밀 운동의 영향

배경 및 목적 림프부종은 방사선 치료와 수술로 야기될 수 있는 만성적인 질환으로, 운동이 이러한 상태에 긍정적인 영향을 미칠 수 있다는 보고가 있다. 하지만, 림프절 절제술과 방사선 치료 이후 운동으로 인한 림프액의 배액 패턴과 같은 림프계와 피부의 변화에 미치는 영향은 아직 명확히 밝혀지지 않았다. 따라서, 본 연구의 목적은 림프절 절제술 및 방사선 치료를 받은 쥐에서 트레드밀 운동 후 림프 배출 경로, 피부 두께 및 섬유증 범위의 변화를 조사하는 것이었다.

방법 Sprague-Dawley 쥐 12마리를 트레드밀 운동과 대조 그룹 각각 6마리씩으로 나누고, 슬와(popliteal)과 사타구니(inguinal) 림프절 절제술을 받은 후 방사선 치료를 통해 림프부종을 유도했다. 운동 그룹은 주당 5일, 30분씩 4주간 트레드밀 운동을 진행했다. Indocyanine green (ICG) 림프촬영을 사용하여 하지를 촬영하고, 매주 양쪽 발목 두께의 차이를 측정했다. 피부 두께, 림프관 밀도 및 섬유증 범위를 조사하기 위해 조직학적 평가도 수행했다.

결과 수술 및 방사선 치료 후 운동은 림프액 배액에 개선 효과를 보였다. 3주차에 ICG 림프촬영 결과, 운동을 한 그룹은 선형(linear) 및 분무(splash) 패턴이 더 많이 나타났다. 또한, 발목 두께는 운동 그룹에서 일관되게 낮았으며, 4주차에서 운동 그룹과 대조 그룹 간의 상당한 차이가 있었다 ($p = .016$). 운동 그룹 쥐들은 대조 그룹 쥐보다 피부의 표피 및 진피가 얇았으며 (각각 $p = .041$

및 .002), 콜라겐 면적 비율이 낮았으며 (p = .002) 림프관 밀도가 높았다 (p = .002).

결론 이 연구에서는, 운동이 림프절 절제술과 방사선 치료와 같은 림프흐름 부전을 유발하는 치료 후 림프계와 피부의 병리학적 변화에 대해 긍정적인 영향을 미칠 수 있음을 시사한다.

색인단어 림프절 절제술; 방사선 치료; 림프부종; 트레드밀 운동; ICG 림프촬영; 쥐 모델