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LONG-TERM FOLLOW-UP OF COLLATERAL PATHWAYS ESTABLISHED AFTER LYMPHADENECTOMY IN RATS

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ABSTRACT

A collateral pathway established after lymphadenectomy could play an important role in long-term lymphedema treatment. The present study investigated alterations of lymph dynamics 1 year after lymphadenectomy using indocyanine green fluorescent lymphography to determine if a collateral pathway may be used for long-term lymphedema treatment. Wistar rats were anaesthetized and lymph nodes were excised at the inguinal and popliteal fossae. The treated hind limb was evaluated by fluorescent lymphography between 10 weeks and 6 months and between 6 months and 1 year postoperatively. Fluorescent lymphography demonstrated a lymphatic pathway to the ipsilateral axillary fossa in all rats 1 year after lymphadenectomy. Some capillary branches in the paths leading to the ipsilateral axillary fossa were dilated and tortuous. In addition, areas in which a fluorescent signal was not visible were increased in the thigh. In conclusion, the collateral pathway did not appear to be only for temporary use to compensate for drainage from the edematous limb but appears more stable as a component of a compensating lymphatic system. These new dilated vessels, although functional at this point, may still be susceptible to disturbance by further alteration to the lymph vessels.

Keywords: lymphedema, ICG, lymphography, collateral pathways, rat, long-term follow-up, lymphadenectomy

Secondary lymphedema is one of the major complications after cancer treatment particularly following surgery and radiation therapy for breast and gynecological cancers. Once secondary lymphedema occurs, daily care for an edematous limb and the skin is necessary for a lifetime. Secondary lymphedema is chronic and non-curable, and protein-rich fluid accumulated in the interstitial space could further cause adipose deposition and tissue fibrosis (1). Moreover, the lymph vessels become fibrotic and lose their normal permeability in the chronic stage (2).

Lymphedema models using animals are fundamental to understand lymphatic mechanisms both in normal and lymphedema and could also serve to substitute for clinical research. These models can particularly play an important role in histologic and molecular studies. Although lymphatic flow patterns using laboratory animals have been visualized in previous studies (3,4), alterations of the flow patterns after lymphadenectomy are not well understood or studied.

We previously produced lymphedema in the rat hind limb similar to human lymphedema and elucidated alterations of lymph flow after inguinal lymph node (LN) dissection for 10 weeks by indocyanine green (ICG) fluorescent lymphography. The result showed that a network-like pattern of the fluorescent signal interpreted as superficial lymphatics around the incision line appeared within three weeks after loss of original lymphatic routes in the hind limb and remained until week 10 postoperatively (5). This network connected to a collecting lymph vessel displayed as a linear signal reaching to the ipsilateral axillary fossa. At the same time, swelling gradually decreased. This finding means that escape routes connecting to nearby lymph nodes through superficial lymphatics are necessary to prevent or minimize edema. The present study sought to determine whether this pathway could be used for long-term lymphedema control and investigated alterations of lymph dynamics after lymphadenectomy using ICG fluorescent lymphography for one year.

METHODS

The protocol to create hind limb lymphedema using a rat has been described previously (5) and the experiments approved by the Animal Experiment Committee of Nagoya University. Briefly, nine male Wistar rats were anesthetized with isoflurane inhalation, and 0.2 ml of a 1 mg/ml solution of ICG was injected subcutaneously into the dorsum of the hindpaw and the medial and the lateral ankle to detect lymph nodes and lymph vessels. After circumferential incision at the right groin, fluorescent lymph nodes (LNs) were excised at the inguinal fossa and the popliteal fossa, and fluorescent lymph vessels were ligated under optical imaging. The skin edges were then sutured end to end.

Evaluation of Limb Edema

The treated hind limb was evaluated by ICG lymphography from Day 3 post-surgery and one year thereafter at the specific periods of time. These time comparisons of fluorescent images were made between week 10 and 6 months, and between 6 months and year 1 after surgery. Indocyanine green lymphography was performed by the same protocol as for surgery. The rat was placed supine for the video recording, and the lens of PDE was set to capture an image from the axilla to the hind toes of the rats. All conditions of the experimental room and excitation light emitted from PDE were controlled. Fluorescent images obtained by PDE were video-recorded on digital format.

RESULTS

From Week 10 to 6 Months

Six months after LNs excision inguinally and popliteally, ICG lymphography shows a linear route connecting to the ipsilateral axillary fossa in all rats, and some rats have a second route to the contralateral inguinal fossa. There is no new route to other parts of the body established between week 10 and 6 months post-surgery, and the routes for each rat has are identical to those observed 10 weeks after surgery.

In all rats, a network-like pattern of fluorescent signals at the treated thigh, the incision line, and the lower abdomen appears to be almost the same as observed at week 10. Furthermore, all rats have no sign of diffuse or spotted fluorescent signals known as dermal backflow. Compared to images taken at week 10, some segments without any fluorescent signals are observed in common.

From 6 Months to 1 Year

One year after LNs excision, ICG lymphography shows a route to the ipsilateral axillary fossa in all rats, and some have an additional route to the contralateral inguinal fossa. There is no new route to other areas created between 6 months and year 1 post-surgery, and the routes for each rat remain the same as those observed 6 months after surgery.

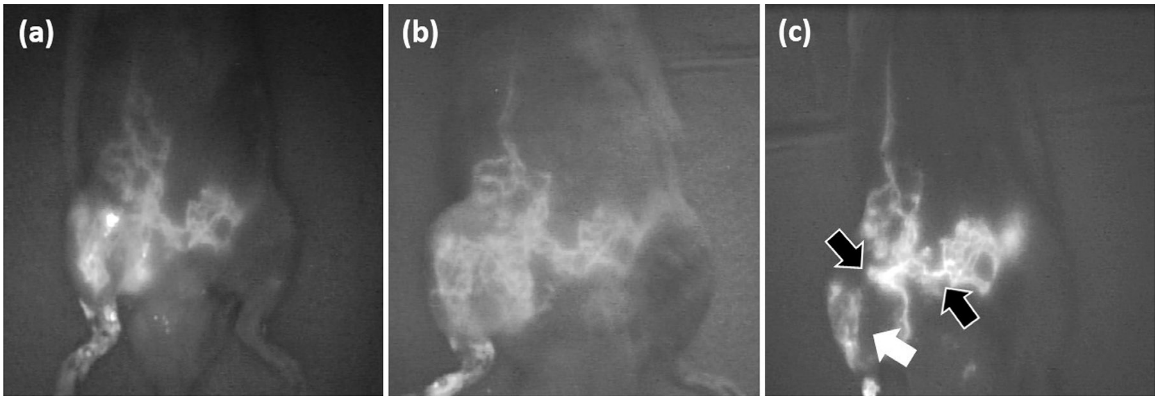

Fig. 1. Collateral pathways from the right hindlimb both to the ipsilateral axillary fossa and to the contralateral inguinal fossa established after lymphadenectomy at the inguinal fossa are depicted by ICG lymphography after (a) 10 weeks, (b) 6 months and (c) 1 year following operation. No differences were observed between (a) and (b) but network signal decrease (white arrow) and some branches became dilated and torturous (black arrow) in (c).

Compared to images taken at 6 months, a decrease of network-like signals which were expanded at the thigh, the incision line, and the lower abdomen is observed. Some capillary branches disappear or blur and in their place dilated and tortuous fluorescent branches are clearly observed in the paths to the ipsilateral axillary and the contralateral inguinal fossa. There are enlarged segments followed by narrow segments on the branches all over the path. In addition, areas in which a fluorescent signal is invisible increase at the thigh as compared to the incision line and the lower abdomen (*Fig. 1*).

DISCUSSION

The present study revealed that collateral routes to the ipsilateral and contralateral axillary fossa developed from the treated hind limb following LN excision in the inguinal fossa after the first postoperative year. In addition, dermal backflow signs were not observed during this period. According to Takeno and Fujimoto, collateral pathways to other LNs have been established within three weeks after LNs excision at the groin and the popliteal fossa (5). After the completion of the pathways, dermal backflow signs gradually decreased and then disappeared completely within 10 weeks after surgery. Thus, it is suggested that these routes are not only for temporary use to compensate for drainage from the edematous limb but may function as a reconstituted lymphatic system. Not all patients who have risk factors for lymphedema develop it in humans. It is considered that those who do not develop it could have such collateral pathways from the limb that possibly develop lymphedema, and this study provides some evidence for this theory. Generally, superficial lymphatic anastomosis is blocked between watersheds on umbilical and midline areas. However, under conditions of increased intralymphatic pressure, the flow of the lymph across the watersheds from one territory to another is facilitated (6). Akita reported an inter-territorial pathway from the edematous limb to the axilla in a patient with chronic lymphedema using scintigraphy (7). In varicose veins, venous valves fail to function and lead to significant reflux under venous hypertension (8). As with lymphatics, excessive intralymphatic pressure could cause valve dysfunction and lead to lymphatic anastomosis between watersheds. Our findings show that network signals, i.e., lymph capillaries, bridge over the incision line from the treated hindlimb to lymph collectors connecting to the ipsilateral axilla. Some capillaries are spread out throughout the body without valves, and formation of these new lymph capillary networks as seen in this present study may contribute to suppression of lymphedema.

Lymph capillaries which were expanded in the treated hind limb, incision line, and the lower abdomen all decreased from 6 months to year 1. In addition, a remaining network-like signal at 1 year was dilated and tortuous compared to images taken six months before. Lymphatic dilation occurs under elevated pressure in lymph vessels (9). Since lymph capillaries have only endothelial cells on the wall and no valves to prevent reflux, increased pressure from the lymphadenectomy may have gradually pushed out the walls over the one-year period. Magnetic resonance imaging of human edematous limbs show that the dilated lymphatic vessels were full of lymph fluid with high pressure in lymphedema (10). In the present study using rats, dilated vessels were observed not in the treated limb but in the collateral pathways between the limb and the target LNs. This finding was first confirmed by a real-time imaging system. In dilated blood vessels, vasoconstriction weakens and blood becomes sluggish or the vessels could not perform a function as the path for circulation. The structure of lymphatic wall and movement of lymph fluid caused by muscle contractions is similar to that of veins. Therefore, the collateral pathways, playing an important role in draining interstitial fluid from the periphery, might be disturbed by further alteration of lymph vessels.

CONCLUSION

This study is the first to reveal alterations of lymphatic flow after lymphadenectomy over one year. We found that LNs excision at the inguinal fossa changed lymphatic drainage routes, and collateral pathways from the treated limb to other LNs were continuously observed at this one-year time point. Therefore, collateral pathways established after lymphadenectomy may be functional for long-term lymphedema control. However, there were enlarged segments on the lymphatic branches all over the path. It is necessary to investigate further how these alterations influence the edematous limb and lymphatic flow. Although these findings were obtained using an animal model, we propose that alterations of lymphatic flow after lymphadenectomy might have a similar course in humans, and further study is needed in this area.

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CONFLICT OF INTEREST AND DISCLOSURE

All authors declare that no competing financial interests exist.

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