Alterations of lymph flow after lymphadenectomy revealed by real time fluorescence imaging system: In vivo study as a baseline for the lymphedema therapy

（リアルタイム画像システムによって評価した
リンパ節切除後のリンパ流の変化
ーリンパ浮腫ケアのための基礎研究−）

名古屋大学大学院医学系研究科
看護学専攻

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平成２５年度学位申請論文

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Abstract

Secondary lymphedema is most often caused by cancer therapy. Conservative treatment, such as combined decongestive therapy (CDT), has been applied to mild or moderate cases in general. However, the mechanism of lymphedema formation and the therapeutic mechanism of CDT and its efficacy are still unclear.

The purpose of the present study is to investigate the lymphedema formation and search the escape route for excess interstitial fluid using 12 rats whose right inguinal lymph nodes were excised. The edematous limb was evaluated by indocyanine green (ICG) lymphography and circumferential measurement. Both evaluations were performed at specific periods of time, from Day 3 for ten weeks after surgery.

Approximately 2 or 3 weeks after lymphedema creation, network-like pattern of the fluorescent signal appeared around the surgical site and became a linear pattern in the lower abdomen. Video-recordings identified fluorescent flow moving from the right lower abdomen in the direction of the ipsilateral axillary lymph node. Circumferential lengths of the treated hind limbs increased, but it decreased as a function of time.

Using these findings in the above mentioned experiment, the second experiment regarding efficacy of CDT was conducted to visualize the changes of lymph fluid accumulating in an edematous limb by the same assessment technique.

Another 12 lymphedema rats in a right hind limb were divided randomly into two groups. On the first day, ICG was injected into an edematous limb of rats, and no-intervention and CDT was applied to groups 1 and 2, respectively, for two weeks. ICG lymphography and circumferential measurements were done every two days.

A fluorescent flow to the ipsilateral axillary fossa was identified in all rats. While manual lymph drainage was applied in the CDT group, the flow moved more rapidly through this pathway than that in the no-intervention group. Furthermore, an area of high-intensity fluorescent signals concentrated around the injection sites diminished in the CDT group more than that in the no-intervention-group after two weeks. Circumferential lengths of the edematous limbs in the no-intervention group showed no

significance decrease during 14 days, whereas that in the CDT group decreased significantly.

The results of the present study add valuable knowledge of lymphedema formation and progression, and offer a promising way to understand an escape route for excess interstitial fluid from an edematous limb.

Abstract (Japanese)

続発性リンパ浮腫は癌治療によって引き起こされることが多い。軽度から中等度のリンパ浮腫の場合には、一般的に複合的治療（CDT）のような保存的療法が用いられる。しかし、リンパ浮腫の形成、CDTの作用機助とその効果については、いまだ不明瞭である。

本研究の目的は、12匹の右鼠径リンパ節を切除したラットを用い、リンパ浮腫の形成と過剰な細胞間質液の排出路を明らかにすることである。浮腫肢の評価については、インドシアニングリーンリンパ管造影法と周囲径測定を用いて行った。両測定方法は、術後3日目から10週間にかけて定期的に行った。

リンパ浮腫形成後 2-3 週間で、創部にネットワーク状の蛍光シグナルが出現し、それが下腹部で線状になるのが認められた。動画で見ると、この線状の蛍光シグナルは右下腹部から同側腋窩に向かって流れていた。浮腫肢の周囲径は術後増大したが、時間の経過とともに減少していった。

これまでの実験から得られた結果を用い、CDT の効果を検証するための第２の実験を施行した。この実験は同じ評価方法を用い、CDT が浮腫に貯留したリンパ液をどのように変化させるのかを視覚化したものである。

最初の実験で用いたラットとは別の右後肢に浮腫が出現した 12 匹のラットを、無作為に二つのグループに分けた。実験初日に ICG を浮腫肢に注射し、グループ１には 2 週間何も行わず、グループ 2 には CDT を 2 週間行った。ICG リンパ管造影と浮腫肢の周囲径測定を、その 2 週間 2 日毎に行った。

グループ１、２のすべてのラットにおいて、同側腋窩へ向かう蛍光の流れが認められた。CDT を施したグループ２のラットに徒手的リンパドレナージを行うと、何も行っていないグループのラットに比べ、リンパ流はさらに速くこの経路を流れた。また蛍注射部位に集積している蛍光シグナルの高輝度に見える範囲は、CDT を 2 週間施したグループ２のラットのほうが、何も行ってないグループ１のラットに比べ縮小した。何も施していないグループ１の浮腫肢の周囲径は 14 日の間に明らかな減少は認められなかったが、CDT を施したグループ２では顕著に減少した。

本研究は、リンパ浮腫の形成と進行を *in vivo* で初めて証明したものであり、研究成果は、浮腫肢からの過剰な間質液の排出路を理解し、さらにリンパ浮腫ケアを開発することに寄与すると考えられる。

Chapter 1 Introduction

The purpose of the present study is to investigate lymphedema formation and progression, and, to search for an escape route for excess interstitial fluid, from the aspect of fluid movement by real time fluorescent system. In addition, new evidences of CDT are sought according to visualizing changes of lymph fluid accumulating in an edematous limb. This preliminary chapter will introduce briefly the broader knowledge of lymphedema. The chapter consists of three sections and a dissertation summary, namely:

1.1 Background of the research

1.2 Significance of the research

1.3 Aims and questions of the research

1.4 Dissertation overview

1.1 Background of the research

Lymphedema is a chronic inflammatory lymphostatic disease caused by mechanical failure of the lymphatic drainage system. There are two types of lymphedema: primary and secondary. The former type is due to a developmental defect of the lymph vessels and/or lymph nodes, and the latter type is mainly caused by cancer therapy performed to prevent tumor metastasis. It could cause a decline in lymphatic transport, and then swelling is produced by accumulation in the extracellular space of excess water, plasma proteins and other cell products (International Society of Lymphology [ISL], 2009).

According to a survey of characteristics of lymphedema in a developed country, the majority of the patients (80 %) had cancer-related lymphedema, and 12 % had non-cancer related lymphedema. Patients with primary lymphedema were only 8 % (Sitzia et al., 1998). Especially, arm edema in women after axillary lymph node dissection for breast cancer is common (Szuba and Rockson, 1998).

There are about 1.38 million new cases of breast cancer each year in major 184 countries (Ferlay et al., 2010). In Japan, there were over 56,000 patients diagnosed as breast cancer in 2007 (National Cancer Center, 2012). Although not all breast cancer patients who take surgery and/or radiation therapy develop secondary lymphedema, it is one of the most troublesome and chronic longterm aftereffects of damage to the lymph nodes (Loprinizi et al., 1999). The incidence rate of lymphedema is estimated that patients with arm lymphedema in Japan would increase from 22,486 people in 2000 to 38,692 people in 2020 using simulation (Kitamura et al., 2005). Thus, the demand for lymphedema treatment is also expected to increase in the decade ahead.

Surgical treatment, for example lymph-venous anastomosis, may be performed for a severe case of lymphedema. On the other hand, conservative treatment, such as complete decongestive therapy (CDT), has been carried out to drain excess fluid from the periphery and been applied to mild or moderate cases in general (Cormier et al., 2012). It consists of four techniques: manual lymphatic drainage (MLD), compression bandage, exercise and skin care. To find its supportive evidences, previous studies have employed various assessment tools which can detect volume reduction of an edematous condition (Koul et al., 2006; Pinell et al. 2008) and improvement of QOL (Mondry et al., 2004). Whereas CDT is likely to obtain the consensus in general, the international society of lymphology states that this method has not yet to undergo sufficient meta-analysis of multiple studies (ISL, 2009). To prove this, CDT has been still uncovered by government insurance in many countries (Towers et al., 2008) including Japan (Matui et al., 2008), and patients with lymphedema can difficultly seek it at low cost in public health service institutions. Therefore, it is sought to examine CDT to create its appropriate protocol which lymphedema patients could access without anxiety.

There is a lack of consensus about clinical criteria of lymphedema for diagnosis and standard methods of assessment (McWayne & Heiney, 2005). Physical assessment, such as circumferential measurement and volume measurement, is fundamentally used to detect swelling of an edematous extremity in the clinical and the research settings, but these convenient methods are indirect and unable to distinguish from whole volume (Smoot et al., 2011). On the other hand, several imaging techniques are available to visualize lymph flow and identify localization of extracellular fluid. However, these assessment methods require full equipped facilities and professional technicians and may cause severe side effects. One of research agenda formulated by ISL is improvement of imaging techniques to allow more precise non-invasive methods to measure lymph flow dynamics (ISL, 2009). Near-infrared fluorescent lymphography using indocyanine green (ICG) is recently used in lymphedema along with the development of photodynamic eye system (PDE: Hamamatsu Photonics K.K.

Hamamatsu, Japan) (Ogata et al., 2007; Unno et al., 2008). The present study used this novel method and tried to investigate lymphedema formation and progression, and to search new findings which could support efficacy of CDT.

1.2 Significance of the research

Reflecting the lack of research regarding efficacy of CDT which almost lymphedema patients need, the present study reveals alterations of lymph flow after lymphadenectomy by real time fluorescence imaging system. There are three major areas of significance to emerge from the present study. Firstly, it is necessary to know how interstitial fluid accumulating in the extracellular space moves from the periphery to the venous system. In lymphedema, pathways lymph fluid returns through have not been understood. Knowledge regarding pathways of lymph fluid is useful to provide lymphedema treatment.

Secondly, studies regarding efficacy of CDT using scientific approaches are very limited. One advantage of real time fluorescence imaging system is that lymph flow can be observed in vivo and the sensitivity to detect it is very high. Using this novel method, it could be possible to visualize the changes of lymph fluid accumulating in an edematous limb. This possibly supports or reverses improved clinical utility of CDT.

Finally, to organize environment for lymphedema treatment, multiple evidences underpinned by strictly-controlled research are absolutely essential. With methods for assessment of lymphedema treatment currently limiting, the present research introduces new one to this field and could provide new fundamental knowledge to continue the next research.

1.3 Aims and questions of the research

The purpose of the present study is to investigate alterations of lymph flow after lymphadenectomy revealed by real time fluorescence imaging system, specifically to reveal:

1. Process and time course of lymphedema formation and progression;
2. Pathways altered by lymphdenectomy in order for lymph fluid to proximally return
3. New evidences of CDT according to visualizing changes of lymph fluid accumulating in an edematous limb

1.4 Dissertation overview

This dissertation consists of five chapters. This chapter has introduced the background on current situations of secondary lymphedema. In addition, issues surrounding lymphedema care have been briefly discussed. Chapter Two will a literature review addressing the themes of secondary lymphedema, differences of incident rates among different approaches, features of assessment techniques, and issues involved in the treatment. These themes are important in understanding the aims and purposes of the present research. Chapter Three will include a discussion of the materials, method, and ethical consideration. Chapter Four will present the results of the experiments. What the results means will be discussed in Chapter Five. Finally, limitations and suggestions for future research are outlined in Chapter Six.

Chapter 2 Literature review

Introduction

This chapter will consider issues concerning lymphedema patients through a literature review of previous research. In Chapter One the present situation of lymphedema patients was introduced, but to understand the issues more deeply, this chapter will extend the area of the literature review. While this dissertation focuses on alterations of lymph flow after lymphadenectomy, the literature review will also examine the research that has been published on the larger context of lymphedema patients.

This chapter is divided into three parts. The first part will explore picture of secondary lymphedema. The second part will discuss diagnosis and assessment methods of secondary lymphedema. The aim of the third and final part is to examine issues surrounding lymphedema treatment.

2.1 Picture of secondary lymphedema

Most cases of secondary lymphedema are due to cancer treatment, such as lymph node dissection and radiation, which is almost routinely performed to prevent tumor metastasis. Among all types of cancer, breast cancer is likely to cause arm lymphedema, and uterine cancer, prostate cancer, or melanoma may cause lymphedema in the legs. Cancer is one of leading causes of death worldwide, therefore not only researchers but also the whole public pay attention to its treatments and prevention. On the other hand, we have very little interest in secondary lymphedema which is the most problematic side-effect.

The incident rate of secondary lymphedema varies among different studies. In upper extremity lymphedema after breast cancer, it is from 5 % to 70 %, and from 1.2 to 47 % in lower extremity lymphedema after pelvic or genital cancer operation. These differences may be influenced by duration of follow-up, types of the primary treatment for breast cancer, and risk factors.

Follow-up phase

Early onset edema develops within the first 2 months while late breast edema occurs about 20 months after surgical and/or radiation therapies (Clarke et al., 1982). Among 263 women taking into consideration categories of lymphedema, 77% of patients reported swelling occurring within three years of diagnosis, and the remaining patients were classified as having late onset lymphedema (Petrek et al., 2001). According to this study, three-year would become a milestone to see the whole picture of secondary lymphedema patients, but patients who have taken the breast cancer therapy likely to get secondary lymphedema anytime. Kissane et al. studied reported that 4.3% of 303 women complained symptoms of lymphedema within the first three months postoperatively despite the changes in surgical and radiotherapy practices (1998). On the other hand, late onset lymphoedema, up to 30 years after radical mastectomy, has been reported (Brennan, 1992).

To summarize the above results, swelling is likely to develop relatively early after the breast cancer therapy. However, patients who possibly develop secondary lymphedema always need to pay attention to the changes of their own health for rest of their lives.

Types of the primary treatment for breast cancer

Approach to treating breast cancer has changed for the last century. Especially, surgical techniques have improved with passing of time from radical mastectomy to breast conservative surgery. Axillary lymph node dissection has been also transmuted into sentinel lymph node biopsy.

Radical mastectomy has been very rare in the current era, but the elderly struggling with arm lymphedema over the years possibly took this procedure. In a report by McDonald, 62.5 % of 40 patients who took radical mastectomy developed secondary lymphedema within several years (1948). By this surgical technique, total mammary gland, pectoralis major and minor muscles, and axillary and supraclavicular lymph nodes are removed, so it is assumed that lymph nodes and vessels could be damaged beyond necessity. Comparing breast-conserving surgery with radical mastectomy, the risk of chronic lymphedema was higher for women who had a radical surgery (Segerstrom et al., 1992). However, even if a less-invasive surgical technique becomes the mainstream, the incident rate of secondary lymphedema after breast cancer surgery is not reduced to zero.

The number of lymph nodes removed is identified as being significant prognostic factors that appear to increase the risk of lymphedema of the arm (Herd - Smith et al., 2001). To avoid dissection of negative lymph nodes, sentinel lymph node biopsy (SLNB) has become the standard for determining lymph node status in early-stage clinically node-negative breast cancer (Rattay et al., 2011). McLaughlin et al. studied the long-term prevalence of secondary lymphedema after SLNB and SLNB followed by lymph node dissection (ALND). At median follow-up of 5 years, lymphedema was assessed by circumferential measurements in 600 women (64%) underwent SLNB alone and 336 women (36%) underwent SLNB/ALND. Of patients undergoing SLNB alone, 5% (31 of 600 women) had measured lymphedema, as compared with 16% (55 of 336 women) of patients undergoing SLNB/ALND.

Although treatment environment patients can participate in is getting to being organized, they cannot judge which treatment is the best by themselves in front of diagnosis of cancer. Choice of treatment for cancer heavily depends on physicians making every attempt to save patients' lives. Therefore, patients and physicians are unable to prevent development of secondary lymphedema completely from necessary treatments. However, patients can reduce risk factors associated with lymphedema by keeping precautions in the daily life.

Risk factors

All patients who take breast cancer surgery and axillary lymph nodes excision do not develop arm lymphedema. To know the patients at high risk for developing lymphedema, various individual and environmental factors have been examined in retrospect. At the moment, several factors existing in the daily life are considered as risks for secondary lymphedema.

Firstly, causal relationships between fat and lymphedema are often dealt with the subject. Helyer et al. examined relationships between arm volume after SLDN or SLDN/ALDN of 137 breast cancer patients and predictors. The result of the study was that the risk of developing postoperative lymphedema was significantly related to the patients' body mass index (BMI). Patients with a BMI >30(obese) had an odd ratio of 2.93 compared with those with a BMI of <25 of having lymphedema (2010). Patients who are originally obese have a higher risk to develop lymphedema after breast cancer surgery, but it is also said that lymph makes fat. Harvey et al. used a new *Prox1*-deficient mouse model of lymphedema that progresses to obesity, and show that leakage of lymph from abnormally formed lymph vessels in these mice stimulates cells to store fat and leads to adult-onset obesity (2005). In this way, obesity is inescapably-tied to secondary lymphedema, so it makes the incident rate of secondary lymphedema increase. Different from types of cancer treatment, lymphedema candidates can make effort to keep their ideal weight.

Other factors, such as patient age, hypertension and handedness and excessive use of limb, may relate to the incidence of secondary lymphedema. Particularly, many lymphedema patients have had experiences of infection. Patients who took breast cancer surgery and axillary lymph nodes dissection are likely to become infected. High incident rates of secondary lymphedema and its risk factors were reported by

Segerstrom et al (1992). One hundred thirty six patients who took breast cancer treatment were investigated in terms of six risk factors. As results, the highest incidence of edema was among patients with a history of one or more infections in the arm on the operated side (89 %). Lymphedema patients are susceptible to become infected because of excision of lymph nodes. Recurrent cellulitis may obliterate sufficient lymphatic vessels in a limb. With re-existing lymphatic hypoplasia, a minimal amount of further damage by cellulitis may trigger lymphoedema. In chronic lymphoedema, skin fissuring and co-existent infections increase susceptibility to cellulitis and to further lymphatic destruction (Robless et al., 2010). Sources of infection which hardly attack a healthy individual could cause infection to patients after lymph node excision. Therefore, prevention of infection reduces the incident rate of secondary lymphedema.

To summarize, secondary lymphedema is terrifying condition that can affect anyone who has taken breast cancer therapy including even axillary biopsy. In addition, it is important to reduce risk factors and take precautions in everyday life prior to the onset of secondary lymphedema.

2.2 Diagnosis and assessment methods of secondary lymphedema

Lymphedema, defined as the abnormal accumulation of protein-rich fluid in soft tissues, results from a dysfunction of the lymphatic system (Foldi & Foldi, 2003). All methods for assessment of secondary lymphedema, used at the moment, cannot be the basis of the definition, although they may contribute to the total body of relevant evidence. A lymphedematous extremity needs to be measured quantitatively at the time of diagnosis and assessed regularly to check response to treatment. Moreover, several imaging techniques are carried out to obtain more specific information of impaired lymphatic flow and/or the typical pattern of abnormal fluid distribution within the tissue in recent days. In this section, issues surrounding assessment methods of lymphedema will be discussed first, and then data from preceding studies using fluorescent lymphograohy is following.

2.2.1 Volume measurement

Most previous studies which explored efficacy of lymphedema treatment used quantitative measurement, such as circumferential measurement or water displacement. These methods are convenient and economical, so not only clinicians and researchers but also patients can easily assess an edematous extremity by themselves. On the other hand, weak points of the quantitative measurement have been sometimes argued in terms of their accuracy and reliability.

Both circumferential measurement and water displacement has several difficulties in measurement, but there is strong correlation between values obtained by these two methods. Tewari et al. compared circumferential volume estimation with water displacement by examining data from 174 upper arms from 87 women. The analysis revealed that there was a highly significant correlation between circumferential and volumetric arm measurements. However, both methods cannot distinguish changes in muscle, adipose, or extracellular fluid volume, or to identify localized areas of swelling (Smoot et al., 2011). Swelling is one of biggest factors which cause functional impairment, a feeling of heaviness and aching, psychosocial maladjustment and psychological morbidity (Mortimer, 1998), so it is important to check volume changes regularly apart from severity of lymphedema.

2.2.2 Imaging techniques

Several types of imaging techniques are used to obtain more specific information from an edematous extremity. However, these tests require full equipped facilities and professional technicians, so all patients at risk for the development of secondary lymphedema cannot always receive them in the clinical settings.

Indocyanine green (ICG) lymphography has been recently developed to provide more rapid imaging of lymphatic function owing to the increased sensitivity associated with higher photon count rates from NIR organic fluorophores over radiotracers (Rasmussen et al., 2009). Sentinel lymph node (SLN) biopsy has been in the spotlight since the 1990s, and dye-guided method has been used to identify lymph node and vessels. ICG is one of agents, and its green color gives visual guide to surgeons. On the other hand, a fluorescent characteristic of ICG has been used for imaging of the retinal and choroidal vasculature for over 30 years (Flower & Hochheimer, 1976). Popularity of sentinel lymph node biopsy and an angiography using ICG for fluorescence labeling in a field of ophthalmology are combined together, and fluorescent-guided method using near-infrared camera was developed in a field of sentinel lymph node biopsy in 2005.

The fluorescence method using ICG, which is usually used for measuring liver function, has recently been applied to the field of lymphography. ICG has the characteristic of exhibiting fluorescence after binding to albumin, both in blood plasma and interstitial space. In the latter case, although ICG combined with albumin cannot be taken into blood capillaries, it drains into lymphatic capillaries, and thereby lymph vessels can be identified by subcutaneous ICG administration. Based on these properties of ICG, lymph flow could be observed transdermally in real-time by the ICG fluorescence imaging Photo Dynamic Eye (PDE) system (Ohnishi et al., 2005). This system can display the inner condition of an edematous region in real time which cannot be detected by quantitative assessment methods (Ogata et al., 2007).

Using ICG lymphography, Yamamoto et al. proposed a new severity staging system which consists of four patters of lymph flow: linear, splash, stardust and diffuse (Unno et al. 2008). Linear is seen in normal and mild lymphedema cases, and the latter three are expressed as dermal backflow patterns based on the progression of lymphedema. In this manner, this novel method has just come into use in a field of lymphology but been still rare to assess lymphedema in the clinical placement. Moreover, it has never been used as an assessment tool to examine the efficacy of CDT.

2.3 Issues surrounding conservative treatment for lymphedema

As early as 1892, Winiwarter suggested the use of lymphatic massage and bandaging to reduce the size of the swollen limb (Mason, 1993). In the 1970s, Földi stressed the importance of skin care, the necessity of eradicating infections, the beneficial effects of compression bandaging, and the need for remedial exercises. He also advocated the

Vodder technique of lymphatic massage that is known as manual lymph drainage (MLD) (Ko et al. 1998). MLD developed by Vodder is different from general massage. The common characteristics of this technique are gentle, predominantly circular stretching of the skin to promote movement of fluid (Foldi & Foldi, 2003). However, there is no literature explaining reasons why this technique can reduce edema volume.

Since then, the idea that CDT is the most effective conservative therapy imperceptibly spread among medical professions and patients without concrete evidence to support its efficacy. Discrepancy between popularity of CDT and the treatment environment not officially recognized is leading several issues.

2.3.1 Barriers of conservative treatment

There is another argument that CDT is time consuming, difficult to perform on regular basis, expensive, and burdensome (Mehrara et al., 2011) because lifetime self-management is required to keep volume of the extremity. Ridner et al. reported difficulties with bandaging and clothing modifications as major problems associated with self-care practice (2011). Bandage compliance improves the reduction of lymphedema volume, but it is difficult to wear the multilayer bandage all day under humid and warm weather and to maintain a normal life (Forner-Cordero et al., 2010).

2.3.2 Lymphedema therapists

There are no government-recognized national standards for lymphedema treatment or training in the U.S. (Medical Advisory Committee (MAC) National Lymphedema Network,

2012) as well as those in Japan. A certification issued by lymphedema therapist course is just voluntary and is not legally-effective. However, without knowledge of anatomy and physiology, skills of drainage and bandage, and communication skill it is difficult to deliver lymphedema treatment to patients.

Armer et al. described a baseline of lymphedema therapist practice based on data submitted by 415 therapists from 46 states in U. S. As a result, most lymphedema therapists (93%) completed over 135 hours of training, but less than half (41%) were certified by the Lymphology Association of North America (LANA) (2010).

It is absolutely necessary to distinguish between lymphedema therapy and general massage, but knowledge and skills taught in the therapist course are not based on concrete evidences.

Among several kinds of medical professions who currently provide CDT to lymphedema patients, nurses can become best lymphedema therapists because special knowledge and skills have been already acquired and they have many situations to work. For example, patients diagnosed as cancer related lymphedema have been already diagnosed as malignancy and have overcome bitter situations, for example hospital stay and surgery. They also spend their days in community while keeping up lymphedema treatment. In these situations, nurses can give important information to prevent onset and aggravation of lymphedema and support them in terms of psychological aspect. In addition, they can early detect abnormality and suggest patients to go to hospitals. Overall, nurses' involvement in lymphedema treatment is relevant.

In 2012, Certification Board for Lymphedema Therapist was established to examine his/her knowledge and techniques regarding lymphedema and certify qualification as a therapist in Japan. Other countries also have similar organizations and programs based on only a result of the volume reducation after CDT without scientific analysis. Before less-evidenced skills spread, it is important to explore lymphedema formation and lymph flow first of all (Kitai et al., 2005) and explore the therapeutic mechanisms of CDT.

2.3.3 Economical aspect of lymphedema therapy

Although the insurance system varies among nations, costs and supplements of CDT are not fully or never covered by public insurance in many countries. A lymphedema patient is exposed to not only physical but also financial burdens.

Towers et al. explored the psychological and social effects of cancer-induced lymphedema using a phenomenological approach. As a result of interviews, all 11 patients and eight spouses strongly expressed financial issues. This study was held under the Canadian context, but this topic was also emerged in Australia (Ryan et al., 2003) and Japan (Matsui et al., 2008). Shih et al. used medical claims data to study an incident cohort of breast cancer patients for the two years after the initiation of cancer treatment. Ten percent of 1,877 patients with breast cancer had one or more medical claims indicating lymphedema. The estimated difference in 2-year costs between those with a lymphedema claim and those without ranged from $14,877 to $23,167, with (Shin et al., 2009). In particular, the major contributors to this cost difference being outpatient visits, prescription drugs, mental health-related services, and diagnostic imaging.

Certainly, lymphedema is a chronic medical condition limiting their activities in the daily lives. However, it is absolutely true that treatment without appropriate evidences is difficultly covered by government insurance.

Chapter summary

This literature review has examined the complex context of secondary lymphedema which focuses on the incident rate, assessment methods and several issues associated with treatment. Lymphedema after cancer therapy is not quite unusual but still mysterious and ambiguous. When considering our professional positions, it cannot be said that we provide evidence-based care even though CDT can work for volume reduction. Nurses have a lot of opportunities to confront lymphedema patients about their treatment, observation, and education, therefore it is important to have knowledge about lymphedema and also consider efficacy of CDT. The studies discussed in this chapter have focused on a number of factors that influence lymphedema patients, but importantly this review highlighted the fact that no study has been done on alterations of lymph flow after lymphadenectomy that could be a baseline of CDT. Therefore, this dissertation will reveal lymphedema formation and pathways altered by lymphadenectomy. The next chapter discusses in detail material and methods used to achieve the research questions.

Chapter 3 Materials and Methods

Introduction

As outlined previously, this dissertation investigates lymphedema formation and progression, and searches for an escape route for excess interstitial fluid, from the aspect of fluid movement. Based on results of these questions, new evidences of CDT are sought according to visualizing changes of lymph fluid accumulating in an edematous limb by fluorescent lymphography.

Considering methodological difficulties and ethical issues to conduct experimental research using humans in lymphedema, scientific evidences which could support lymphedema treatment are lacking in clinical practice. However, conservative care methods, especially CDT and MLD, are routinely used for many lymphedema patients, and knowledge and skills of them might be handed down from one generation to another without accurate examination. Therefore, it is sought to examine lymphedema formation and its treatment by different approach so that medical professions could offer well-supported care to patients.

This chapter in particular consists of five major sections: 3.1 Materials; 3.2 Ethical consideration; 3.3 Surgical Procedures; 3.4 Experimental schedules; and 3.5 Evaluation of limb edema and lymph flow.

3.1. Materials

Twenty-four male Wistar rats were used in the present study. The rats were purchased from Japan SLC Company (Hamamatsu, Shizuoka, Japan) and housed in groups of 2 per cage. Room temperature and humidity was maintained at 26 °C and 40-60 %, respectively, and automatic light control was set on 12:12-hr cycle. The rats could access to food and water with ad libitum.

3.2. Ethical consideration

All *in vivo* experiment protocols were reviewed and approved by the Animal Experiment Committee of Nagoya University (Approved number 024-001).

All experiments were conducted in accordance with Related Laws and Regulations.

3.3. Surgical Procedures

To create a lymphedema rat, a surgical method employed in the present study was based on the previous research and our pre-test. All the procedure was done under general anesthesia. A rat was set in a separate box and initially anesthetized by 4% isoflurane inhalation as an introduction. After confirmation of no reaction, it was lifted out from the box, worn a special mask, and were maintained with approximately 1.6% isoflurane throughout surgery. Bilateral hind limbs were shaved in order to measure the circumferential length at both groins. Then, 0.2 ml of ICG solution (1 mg/ml) or 0.2ml of solution that ICG and Evans blue (EB) dye (100 mg /ml) was mixed in equal amount was injected subcutaneously into the dorsum of the right paw and both the medial and lateral ankle to detect lymph nodes in the groin and lymph vessels in the femoral area.

Normal lymph flow and lymph nodes were easily confirmed by photodynamic eye system (PDE: Hamamatsu Photonics K.K. Hamamatsu, Japan), which activates ICG by emitting light at the center wavelength of 760 nm or under an optical imaging. To block lymph flow from the right hind limb, we employed two ways to make an incision.

Method 1: a circumferential incision at the right groin

Method 2: an incision along the right inguinal ligament

In both methods, an incision was made from a surface of skin to subcutaneous tissue. After the skin was denuded from fascia, lymph nodes and lymph nodes with surrounding adipose tissue in the right groin and the ipsilateral popliteal fossa were completely removed. Fluorescent or dyed lymph vessels were carefully ligated under an optical imaging by 10-0 monofilament non-absorbed suture. Furthermore, a dyed lymph node in the pelvis was excised in method 2. Finally, the skin edges were sutured end to end by 4-0 nylon. No dressings or topical treatments were applied to any of the wounds.

3.4 Experimental schedules and evaluation of limb edema

To seek pathways altered by lymphadenectomy, surgery was done by method 1 (circumferential incision) in 12 rats. Evaluations by ICG lymphography and circumferential measurement were performed at specific periods of time from the third postoperative days for ten weeks under general anesthesia.

For ICG lymphography, 0.2 ml of ICG was injected into the same sites as those used on the day of the surgery, and observations were performed by PDE in order to examine the dynamic image of lymph flow patterns. However, ICG was not injected if dermal back flow was already confirmed in the hind limb before the injection. Fifteen minutes after the injection, fluorescent images visualized by PDE were video-recorded as digital format.

To measure limb circumferences, a soft plastic tape measure, used for a human's finger, was used. The incision line at the right groin and the same legion at the left groin which was marked by oil-based ink were always measured. Differences between the right and the left hind limbs were considered as limb edema and its time-dependent change was recorded in a graph.

Bilateral differences of the lengths are presented as medians and ranges. The significance of differences was assessed with the non-parametric Wilcoxon single rank test with Bonferroni correction. Values of $p<0.05$ were considered significant.

Using results obtained by the above-demonstrated experiment, the second experiment regarding efficacy of CDT was conducted in another 12 rats whose surgery was performed by method 2 (incision along with right inguinal ligament). Five weeks after lymphadenectomy, the rats were divided randomly into two groups as figure 1. An experiment protocol of both groups was consisted of three parts: two-week-observation, one-week-interval, and following two-week-observation. On the first day of each two-week-observation period, 0.2 ml of a 1 mg/ml of ICG was injected into the same spots used for when lymph nodes and vessels were dyed by EB. Then, observation by PDE was performed. Circumferential lengths were also measured at the proximal site of the incision line and the untreated contralateral thigh bilaterally with a soft plastic tape measure. ICG lymphography and a circumferential measurement were done every two days in each two-week-observation.

Group 1 (n=6)

No intervention was applied in the first two-week. After the one-week-interval, CDT was applied in the next two-week.

Group 2 (n=6)

Combined decongestive therapy was firstly applied in the first two- week. After the one-week-interval, no intervention was applied in the next two-week.

Procedure of CDT

Compression decongestive therapy which consisted of manual lymph drainage (MLD), compression bandage, exercise and skin care was applied. Regarding MLD, only stationary circle for 15 minutes was performed to the treated hind limb, the bilateral groins, right lateral side of the abdomen toward ipsilateral axillary region from right inguinal ligament. Bandage compression was accomplished using cotton bandage for a under layer and 1 cm–wide adhesive elastic bandage for overlapping layers in proceeding proximal direction from the toe to the thigh. The rat with compression bandage was released into the cage and spent for two days. The rats vigorously moved to feed ad libitum. Such moving in a cage was considered as exercise. Skin condition was checked every two days.

Analyze of data

The images visualized by ICG lymphography were video-recorded and compared between the pre- and each observation period. Differences of circumferential lengths between a treated and an untreated hind limbs were expressed as means ± SDs. The significance of differences was assessed with the one-way repeated measure ANOVA (analysis of variance). Values of $p<0.05$ were considered significant.

Chapter summary

This chapter outlined method and strategy for collecting the data in the present study. It was found that the experimental research suited the present study best when trying to reveal new aspects of lymphedema using a novel assessment method. Further, the animal experimental study was considered to be the most appropriate for the purpose of the present study, as it was able to intervene during the study and administer chemical agents. The first experiment was followed by the second experiment. Data obtained by ICG lymphography were expressed in detail and those by circumferential measurements were statistically analyzed. In the next chapter all results are presented.

Chapter 4 Results

Introduction

This chapter presents the data obtained through two kinds of experimental procedures with lymphadenectomy rats. The research questions were as follows: firstly, process and time course of lymphedema formation and progression; secondary, pathways altered by lymphdenectomy in order for lymph fluid to proximally return; and thirdly, new evidences of CDT according to visualizing changes of lymph fluid accumulating in an edematous limb.

The experiment had two kinds of procedures but results are categorized into three parts:

4.1 Preoperative image

4.2 Postoperative lymphedema formation and alterations of lymph flow

4.3 Efficacy of CDT in an edematous limb

Each part has been divided into sub-headings.

4.1 Preoperative image

All rats showed the same fluorescent image before surgery (Fig. 2). On the right foot pad including the injection sites, intense fluorescence of ICG was observed by PDE. From the right ankle, two linear fluorescent signals immediately appeared after the injection, and then reached the right popliteal fossa and the inguinal region, respectively.

No fluorescent signal was observed on the proximal area of the inguinal region, nor on the other parts of the body except for the right hind limb.

4.2 Postoperative lymphedema formation and alterations of lymph flow

Lymph node excision and lymph vessel ligation at the inguinal region altered the fluorescent patterns, which were observed before surgery in different patterns. Results of the present study are categorized chronologically into three events: 4.2.1 Abnormal images limited to the limb; 4.2.2 Appearance of new flow patterns; and 4.2.3 Stability of flow pattern. Then finally, 4.2.4 Results of circumferential measurements and 4.2.5 Statistical analysis of lengths are following.

4.2.1 Abnormal images limited to the limb

The fluorescent signal did not show a linear pattern in any parts of the treated hind limb, but was distributed diffusely and/or made spotted fluorescent signals in all cases (Fig. 3). Such abnormal images were limited to the treated hind limb.

4.2.2 Appearance of new flow patterns

As new flow patterns, network-like and linear fluorescent signals appeared in all the rats. Such patterns appeared at week 1 in two of twelve rats, week 2 in five rats, and week 3 in five rats after surgery, respectively.

A network-like pattern of the fluorescent signal was dense around the surgical site from the anterior thigh to the lower abdomen. In addition, the fine and small network–like signal was collected at one or several points of the lower abdomen and became a linear pattern. All lines led to and reached the ipsilateral axillary fossa and accompanied superficial epigastric and thoracoepigastric veins. With moving pictures by PDE, the fluorescent flow which moved from the lower abdomen toward the axillary fossa was identified. In nine of twelve rats, the network-like pattern of fluorescent signals was also observed to pass transversely through the suprapubic region and then reach the contralateral inguinal fossa. A linear pattern was not seen in this part. The diffuse and spotted fluorescent signals remained in the anterior thigh even though the network-like and linear flow patterns appeared (Fig. 4). In the gluteal area and the dorsal region of the trunk, the network-like fluorescent signal was barely or never seen.

4.2.3 Stability of flow pattern

All the rats showed a new route to the ipsilateral axillary fossa beyond the incision line. When the new flow patterns began to appear, three rats had a single route to the ipsilateral axillary fossa, and in addition, the remaining rats had another route to the contralateral inguinal fossa. However, the route to the contralateral inguinal fossa disappeared in three rats during observation. The disappearing time of the route to the contralateral fossa in each rat was at week 2, week 5, and week 8 after surgery, respectively. As a result of adding three rats which had only a single route to the ipsilateral axillary fossa from the beginning, at least a total of six rats had a single route to the axillary fossa at the end of the observation (Fig. 5). Regarding the network-like pattern and the route to the ipsilateral fossa, there were a few variations among the rats, but each rat maintained one’s specific flow patterns at least until the end of the observation. Diffuse and spotted signals decreased with the passage of time, since the flow patterns were stabilized and completely disappeared around eight weeks after surgery in all rats. The network-like pattern at the lower abdomen and the linear pattern to the axillary fossa had been seen continuously, at least until the end of observation. Less fluorescent signals still remained in the gluteal and dorsal regions of the trunk, compared to the anterior thigh and the abdomen (Fig. 6).

4.2.4 Circumferential measurements

Preoperative circumferential lengths of the right hind limbs were almost the same as in the left hind limbs in all rats. The median difference in the lengths was 0 cm (from -0.1 to 0.2) in the preoperative period.

Circumferential lengths of the treated hind limbs increased soon after surgery and achieved peak lengths as follows: the period of maximum difference between the lengths of treated and untreated hind limbs was on day 3 for three rats, week 1 for five rats and week 2 for two rats. However, only two rats, whose maximum circumferential lengths occurred in week 2, showed less or the same circumferential length in the right hind limb compared to the left, from day 3 for two weeks. The medians were 0.75 cm (from -0.2 to 1.9) on day 3 and 0.9 cm (from -0.2 to 2.6) on week 1, 0.55 cm (from -0.1 to 1.8) on week 2, respectively.

From week 3 to 10, there was a considerable variation in the changes in circumferential lengths among rats. In some rats, it showed a rapid reduction after the peak and plateau at approximately the same lengths, but a mild reduction continued to be close to those of the untreated hind limb in others. From week 3 to 10, the median difference decreased from 0.4 cm (from -0.4 to 1.2) to 0.1 cm (from -0.2 to 0.6).

4.2.5 Statistical analysis of lengths

The results of statistical analysis revealed significant differences between the pre-operative length and day 3, and between the pre-operative length and week 1 ($p<0.05$). After week 3, there were no significant differences (Fig. 7).

4.3 Efficacy of CDT in an edematous limb

Based on results of preceding study regarding lymphedema formation and alterations of lymph flow after lymphadenectomy, efficacy of CDT were sought according to visualizing changes of lymph fluid accumulating in an edematous limb. All data obtained by this trial experiment is divided into three parts to deliver results; 4.3.1 Group 1, 4.3.2 Group 2, and 4.3.3 Comparison between group 1 and 2. In the final part, the first-2-week-period of the both groups, no intervention for group 1 and CDT for group 2, was compared.

4.3.1 Group 1

No intervention period

On the day of ICG injection, a high-intensity fluorescent signal was concentrated around the injection sites (Fig. 8a). In addition, network-like and dermal backflow patterns were identified in the lower legs and thighs 15 minutes after ICG injection. A fluorescent flow which moved from the lower abdomen toward the ipsilateral axillary fossa was observed in all the rats. A circumferential length of the treated hind limb was longer than that of the untreated limb in all rats. The mean difference between the limbs was 1.9 ± 0.54 centimeter (cm) .

On day 2, the fluorescent pattern in the treated hind limb changed slightly compared to the day of ICG injection. The concentration of the fluorescent signal around the injection site was almost same as that on the day of ICG injection, except that the network-like pattern observed on the lower leg and thigh had disappeared. Instead, a diffuse-like fluorescence signal was distributed unevenly in the whole of the treated hind limb. No fluorescent flow to the ipsilateral axillary fossa was detected.

From days 4 to 14, fluorescent images were almost identical to those on day 2 (Fig. 8b).

The mean difference in circumferential lengths between the limbs was reduced for 14 days to 1.5 ± 0.41 cm. There were no significant differences between the day of ICG injection and days 6, 10, and 14 (Fig. 8).

CDT period

On the day of ICG injection, a high-intensity fluorescent signal was concentrated around the injection sites (Fig. 8c). In addition, network-like and dermal backflow patterns were identified in the lower leg and thigh 15 minutes after ICG injection. A fluorescent flow to the ipsilateral axillary fossa was also observed. While MLD was applied, the flow moved rapidly along this pathway. However, the area of the fluorescent signal in the treated hind limb did not noticeably change before and after MLD by PDE observation. The mean difference in circumferential lengths between the limbs was 1.7 ± 0.17 cm.

On day 2, the fluorescent pattern in the treated hind limb changed slightly compared to the day of ICG injection. The concentration of the fluorescent signal around the injection site was almost the same as on the day of ICG injection, but the network-like pattern observed on the lower leg and thigh had disappeared. Instead, a diffuse-like fluorescence signal was distributed unevenly in the whole of the treated hind limb. The route to the ipsilateral axillary fossa was blurry but still observable after MLD was applied.

Unlike the no intervention period, the fluorescent signal around the injection sites diminished gradually during the day from 4 to 14 (Fig. 8d). On day 14, the mean difference among the limbs was 0.35 ± 0.14 cm. There were significant differences between the day of ICG injection and days 6, 10, and 14 (Fig. 8).

4.3.2 Group 2

CDT period

On the day of ICG injection, a concentration of the fluorescent signal around the injection sites was observed (Fig. 9a). A high-intensity fluorescent signal was distributed from the foot pad to the ankle. In the lower leg and the thigh, network-like and dermal backflow patterns such as stardust were observed for least 15 minutes. A fluorescent flow that moved from the lower abdomen toward the ipsilateral axillary fossa appeared in all the rats. A circumferential length of the treated hind limb was notably longer than that of the untreated limb. The mean difference between the limbs was 1.62 ± 0.32 cm. On day 2, the concentration of the signal around the injection site was almost the same as on the day of ICG injection, but the dermal backflow patterns on the lower leg and thigh had disappeared. Instead, a fluorescent signal was distributed diffusely and unevenly in the whole of the treated hind limb. The shape of the route to the ipsilateral axillary fossa was ambiguous.

On day 14, the fluorescent signal around the injection sites had decreased in comparison with day 2 (Fig. 9b). The mean difference in the lengths also diminished to 0.27 ± 0.2 cm. There were significant differences between the day of ICG injection and days 6, 10, and 14 (Fig. 9).

Interval

A difference in circumferential lengths increased during one week. After a *t*-test, the difference was significant one (Fig. 9).

No intervention period

On the day of the ICG injection, a concentration of the fluorescent signal around the injection sites was observed (Fig. 9c). A high-intensity fluorescent signal was distributed from the foot pad to the ankle. In the lower leg and thigh, network-like and dermal backflow patterns such as stardust were observed for least 15 minutes. A fluorescent flow which moved from the lower abdomen toward the ipsilateral axillary fossa appeared in all rats. A circumferential length of the treated hind limb was longer than that of the untreated limb. The mean difference between the limbs was 0.93 ± 0.31 cm.

The concentration of the fluorescent signal around the injection sites changed little for 14 days in comparison to that in the CDT period (Fig. 9d). The mean difference in lengths between the limbs increased slightly to 1.05 ± 0.31 cm in comparison with the day of the ICG injection. However, there were no significant differences between the day of the ICG injection and days 6, 10, and 14 (Fig. 9).

4.3.3 Comparison between groups 1 and 2

During the first two-week period of both groups, no intervention for group 1 and CDT for group 2 was compared.

The fluorescent images observed on the day of ICG injection were almost identical between groups. The fluorescent flow to the ipsilateral axillary fossa was identified in all rats. However, while MLD was applied in group 2, the flow moved more rapidly through this pathway than that in group 1.

The fluorescent signal concentrated around the injection sites decreased for 14 days in groups 1 and 2. However, a weaker signal was detected in the CDT group (Fig. 9a, 9b) than that in the no-intervention group (Fig. 8a, 8b).

Circumferential lengths of the treated hind limb were longer than untreated limbs in both groups 1 and 2 on the day of ICG injection. Group 1 (no intervention) showed no significance differences during 14 days, whereas group 2 (CDT) exhibited very significant differences.

Chapter summary

A lot of new findings were revealed from the major and the trial experiments. Some of results sustain a part of MLD treatment and CDT therapy. However, the present study highlights several findings in lymphedema formation and progression, pathways altered by lymphadenectomy, and changes of lymph fluid accumulating in an edematous limb. These new findings will be taken up the next chapter as discussion.

Chapter 5 Discussion

Introduction

The previous chapter exhibited results categorized into three parts in detail. This chapter presents discussion of each result in relation to the literature.

5.1 Postoperative lymphedema formation and alterations of lymph flow

In the present study using the PDE system, lymph node excision and vessel ligation at the inguinal site appeared to lead to the development of collateral pathways to the ipsilateral axillary and contralateral inguinal lymph nodes. Dermal backflow signs considered as lymphedema appeared in the treated hind limbs and then gradually disappeared after the collateral pathways were established. Therefore, collateral pathways are presumed to have a crucial role in improving lymphedema and for compensating for lymphatic circulation from a lower extremity. In particular, the pathway to the ipsilateral axillary lymph nodes may have a large potential for allowing lymph fluids to drain into the deep lymphatic system, because lymph flow movement toward the ipsilateral axillary lymph nodes was continuously observed despite the disappearance of the suprapubic pathway. This was first confirmed as real-time lymph movement using the PDE video-recording system. Lymph labeled by ICG was observed by film to **vigorously** move from the paw, beyond the incision and to the ipsilateral axilla. Recently, dermal back flow was observed in lymphedema patients (Unno et al., 2008). Considering these facts and our findings using PDP video, it is possible that the collateral pathway to the ipsilateral axillary lymph node may be activated in patients suffering from lower extremity lymphedema. This knowledge is considered to be very beneficial.

Superficial cutaneous lymph capillaries seen as network-like patterns were observed from the thigh to the lower abdomen beyond the incision. Therefore, it is supposed that they also play a greater role in connecting the treated hind limb and the collateral pathway. Several studies found that the maximum spread of the visualized microlymphatic network was significantly larger in patients with lymphedema than in healthy controls (Bollinger, Jager & Sgier, 1981; Mellor et al., 2000), because of an impeded transport toward the deeper channel. However, these studies refer only to the capillary network from the injection site. In the present study, a spread of the superficial lymph network crossing the incision line was observed. We emphasize that lymph capillaries regenerate and/or connect to each other, and spread to beyond the affected region where the lymph transportation system is damaged. The capillaries might reach adjacent normal lymph collectors and then drain into normal lymph nodes.

The present study employed circumferential measurements in parallel with ICG lymphography. The statistical analysis shows that there are significant differences in the median difference of the circumferential lengths between the preoperative period and day 3, and between the preoperative period and week 1. This may coincide with the stage in which that abnormal images are limited to the treated limb. The decline from week 2 could overlap the stage of the appearance of new flow patterns. Although severe dermal backflow patterns appeared in the treated right hind limbs of all rats, two rats showed less or almost the same circumferential length in the right hind limbs compared to that in the untreated left hind limbs until week 2. The treated right hind limbs gradually swelled, and then showed the same lengths as those in the left hind limbs at week 1 and 2 after surgery, respectively. The circumferential lengths of the treated hind limbs, which were shorter than the untreated hind limbs until one week after surgery, may have been influenced by the removal of adipose tissue at the time of surgery, so the onset of swelling might be delayed in comparison with the emergence of dermal backflow. Although apparent swelling of the treated hind limbs was not identified until two weeks after surgery, ICG lymphography revealed significant changes in lymph flow during this period. A similar finding has also been reported and discussed by Ogata et al. (2007). They found that swelling of the treated limb measured by circumferential lengths was not apparent until the day 4 or 5 after lymph node resection, but that the ICG lymphography revealed significant changes in lymphatic vessels even at day 3. Therefore, for the early detection of lymphedema, it is important to not depend exclusively on visible swelling. ICG lymphography may improve clinical practice greatly. In Ogata et al.'s study, only acute lymphatic damage from the preoperative period to day 7 post-surgery was studied. We furthermore pursued lymphatic changes and swelling for 10 weeks. The results of the present study add valuable knowledge of lymphedema formation and progression, and offer a promising way to understand an escape route for excess interstitial fluid from an edematous limb.

5.2 Efficacy of CDT in an edematous limb

The fluorescent signal presented with a network-like pattern in the treated hind limb travelled beyond the incision line, where it became a linear pattern at the right lateral region of the abdomen reaching to the right axilla. The network-like and linear patterns represent lymph capillaries and collectors, respectively. Therefore, lymph fluid that drained from the periphery by CDT moved through lymph capillaries, flowed into lymph collectors, and then reached the axillary LN. A reduction of an edematous limb by an application of CDT has been well-documented (Koul et al., 2006; Pinell et al., 2008). However, pathways of lymph fluid and destination LNs have never been studied scientifically. The present study revealed drainage routes of lymph fluid in a lower limb lymphedema.

The PDE system captured the high-intensity area of a fluorescent signal around the injection site after ICG was injected into the subcutaneous space of the right paw and ankle. After CDT was applied for two weeks, the high-intensity area at the injection site was reduced, and the low-intensity fluorescent signal was increased instead. These changes indicated that the application of CDT to the edematous limb encourages lymph fluid to move from the periphery. This is the first demonstration that the efficacy of CDT has been visualized. ICG is less invasive, and its fluorescent navigation is very sensitive. The detection rates of sentinel LNs and lymphatic channels are 94~97% (Kitai et al., 2005; Hirche et al., 2010) and 100% (Kitai et al., 2005; Abe et al., 2011), respectively.

In both groups, the high-intensity area of the fluorescent signal was reduced after the 2-week-CDT, but there was little reduction without CDT for another 2 weeks. The changes in fluorescent images were well-supported by the changes in circumferential lengths, thus rendering the evidence becomes clearer than ever before. Each assessment method has several weak points. The present study offers a new experimental method that compensates for the weaknesses of CDT research.

Chapter summary

Through the experimental results, new and valuable knowledge of lymphedema formation and progression, and escape routes for excess interstitial fluid from an edematous limb were revealed. In addition, efficacy of CDT was visualized by observing changes of lymph fluid accumulating in an edematous limb.

First of all, instead of normal lymph flow from the periphery, lymph fluid intends to temporarily flow through lymph capillaries which do not directly connect to lymph nodes into other lymph collectors, not used under normal circumstances. In a rat, a collateral pathway between inguinal and axilla regions is often used if the inguinal lymph node is damaged.

Second, increase and decrease in the circumferential lengths during 10 weeks may coincide with the stages of the appearance of the fluorescent pattern. Although apparent swelling of the treated hind limbs was not identified, ICG lymphography revealed significant changes in lymph flow. Therefore, for the early detection of lymphedema, it is important to not depend exclusively on visible swelling.

Next, application of CDT for two weeks encourages lymph fluid to move from an edematous limb. This is the first demonstration that the efficacy of CDT has been visualized.

Finally, the changes in fluorescent images were well-supported by the changes in circumferential lengths, thus rendering the evidence becomes clearer than ever before.

In the final chapter, recommendation and suggestions for future research will be stated.

Chapter 6 Conclusion

Introduction

The previous chapter picked up and discussed findings in detail. This chapter presents a number of limitations and suggestions for future research before stating final conclusion. The present study focused on how lymph flow from the periphery would be altered after the inguinal lymph node was excised. A number of findings are very linked to the establishment of collateral pathways. The following recommendations from the present study and suggestions for future research may be useful for medical professions involved in lymphedema treatment and for patients struggling with lymphedema.

6.1 Limitations of the present study

Several methodological limitations are identified to the present study.

- Species limitations existed. The present study extrapolates the results of animal experiments to humans.
- Procedures of lymphedema creation in the present study did not correctly reflect those of breast cancer or pelvic cancer surgery for humans. Extensive excision of lymph nodes was performed this time.
- The number of rats used in the present study is not large. Statistical analysis was employed to seek a significant difference in circumferential lengths during observation periods, but there might be slight errors.

6.2 Suggestions for future research

Suggestions for future research arise mostly from results from the present study not previously discussed in the literature on lymphedema research.

Intervention study in humans

This experimental study analyzed data form lymphedema rats, so further study is required using human subjects. This may make it possible in the future to conduct a clinical intervention study to assess the complex condition of lymphedema.

Longitudinal study

The present study collected data for 10 weeks after lymph nodes were excised. A longitudinal study may bring more information to add knowledge regarding lymphedema.

Histological and immunohistological examination

To deeply understand that lymph flow moved from the lower limb toward the ipsilateral axillary lymph node beyond the watershed, it is not enough only to observe lymph flow by ICG lymphography, so histological and/or immunohistological examination study is required.

Conclusion

The present study aimed to investigate alterations of lymph flow after lymphadenectomy revealed by real time fluorescence imaging system. Complete decongestive therapy has been widely carried out to reduce volume of an edematous extremity, but evidences obtained by scientific research have been still lacking. The present study revealed lymphedema formation and progression, and escape routes for excess interstitial fluid. Findings revealed by the present experimental study are followed.

1. After lymph node excision and vessel ligation at the inguinal site, collateral pathways to the ipsilateral axillary and contralateral inguinal lymph nodes were developed. Then, edema reduction was observed.
2. Network-like fluorescent signal was observed around the incision. Lymph capillaries were observed around the incision. They play a greater role in connecting the treated hind limb and the collateral pathway.
3. Fluorescent patterns observed by ICG lymphography did not necessarily correspond to results obtained by circumferential measurements.
4. CDT was effective to encourage lymph fluid to drain from the edematous limb.

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