Lymphangiogenesis
A Potential New Therapy for Lymphedema?

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At the level of the capillaries, the systemic circulation loses ∼2 to 4 L fluid and ∼100 g protein into the interstitium daily. This ultrafiltrate of the systemic capillaries is returned to the circulatory system by the lymphatics. The lymphatic vasculature is highly specialized to perform this service, beginning with the blind-ended lymphatic capillaries. These vessels are highly permeable to protein, fluid, and even cells because of fenestrations in their basement membrane and discontinuous button-like junctions rather than tight intercellular junctions, as observed in the systemic capillaries.1 The lymphatic capillaries merge into collectors and intercellular junctions, as observed in the systemic capillaries, and form larger lymphatic conduits that are invested with vascular smooth muscle (capable of contracting and propelling lymph forward) and valves for unidirectional flow. These conduits merge at lymph nodes, delivering antigens to the immune cells and serving as an early warning system of pathogen invasion. The lymph nodes drain into conduits that ultimately merge into the thoracic duct, which empties into the left subclavian vein.

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Lymphedema may be secondary to acquired obstruction of the lymphatics (as with surgery, tumor, trauma, or infection). The most common cause of secondary lymphedema in developed countries is breast cancer and its treatment.2 In men, prostate cancer and lymphoma are common causes. In contrast, in primary lymphedema, the lymphatics are congenitally hypoplastic or maldeveloped. A number of mutations of genes involved in lymphatic development are associated with primary lymphedema (GJC2, FOXC2, CCBE1, VEGFR-3, PTPN14, GATA2, and SOX18).3 Developmental and genetic studies have shown that lymphangiogenesis requires the C isoform of vascular endothelial growth factor (VEGF), which binds to its receptors, VEGFR-2 and VEGFR-3. The expression of these receptors on lymphatic endothelial cells during development is under the control of the transcriptional factor Prox1, the master regulator of lymphangiogenesis.4 This increased understanding of lymphangiogenesis has led to preclinical trials of VEGF-C augmentation that seem promising.

A Novel Lymphangiogenic Therapy?

Accordingly, the article by Choi and colleagues5 arrives at a propitious time. The authors provide evidence that 9-cis retinoic acid (9-cisRA), a naturally occurring retinoid that binds all known intracellular retinoic acid receptors,6 is capable of inducing lymphangiogenesis in preclinical models. This is an intriguing observation, because 9-cisRA is approved by the Food and Drug Administration for topical application in Kaposi sarcoma;6 because there is considerable clinical experience with this drug, development and entry into clinical trials could be expedited. However, before launching into our clinical trial, let us take a closer look at the evidence.

To begin, Choi and coworkers observed that 9-cisRA and other retinoid acid derivatives increased the proliferation, migration, and tubulogenesis of human lymphatic endothelial cells. This is surprising, given that 9-cisRA has been used to treat Kaposi sarcoma, a form of pathological endothelial proliferation, and that 9-cisRA and other retinoids have antiproliferative effects.8,9 It is worth noting that the investigators may have seen a biphasic effect of the agent if higher doses had also been studied.

In any event, the investigators hypothesized that these surprising effects of 9-cisRA might be mediated by angiogenic growth factors. VEGF and fibroblast growth factor (FGF) are known endothelial mitogens, so it was reasonable to assess the effects of pharmacological antagonists of their action. Whereas 2 different antagonists of the FGF receptor (FEFR) blocked the effects of 9-cisRA, antagonists of the VEGFR-2 or VEGFR-3 receptors had no effect. Furthermore, lymphatic endothelial cells treated with 9-cisRA were observed to manifest a transiently increased expression of FGF receptors (FGFR3 and FGFR4). Finally, a soluble form of FGFR3 also blocked the effect of 9-cisRA on lymphatic endothelial cell proliferation (presumably by scavenging released FGF). So, it seems reasonable to conclude that the effects of 9-cisRA, at least at lower doses, are mediated by FGF. This finding also raises a concern, discussed in more detail below.

In addition, Choi et al observed that 9-cisRA downregulated the expression of 2 cell-cycle inhibitors, p27 (CDKN1B) and p57 (CDKN1C), and increased the expression of 2 aurora kinases, the activity of which promotes cell cycle. Further studies suggested that these effects of 9-cisRA could be mediated by nongenomic and genomic mechanisms. Specifically, the downregulation of p27 was due in part to its phosphorylation by Akt kinase (which is known to result in the degradation of p27). In contrast, the downregulation of p57 was on a genomic basis, mediated by the transcriptional
factor Prox1, which is known to up-regulate p57. As expected, overexpression of Prox1 increased p57 expression, and down-regulation (by siRNA) of Prox1 had the opposite effect. Exposure of the lymphatic endothelial cells to 9-cisRA caused Prox1 to dissociate from p57 (as detected by chromatin immunoprecipitation assay). Additional studies (including combined knockdown of Prox1 and the nuclear receptor of retinoic acid [RXRα]) indicated that 9-cisRA is likely acting on additional cell-cycle proteins and that its effects may be mediated through retinoic acid receptors other than RXRα. In conclusion, 9-cisRA had nongenomic and genomic effects on multiple cell-cycle proteins, explaining in part its effects on lymphatic endothelial cells.

Finally, the investigators used 4 preclinical models to demonstrate the lymphangiogenic effect of 9-cisRA. After 5 days of intranasal application of 9-cisRA or vehicle, mouse trachea tissue manifested increased lymphatic vasculature. In a second model, Matrigel was placed subcutaneously in mice, forming a plug that was harvested 2 weeks later. Those plugs, which contained 9-cisRA, displayed more prominent lymphatic capillaries, as detected by staining for the lymphatic marker podoplanin. Similar results were observed in the corneal micropocket assay. Most significant were their results with the mouse tail lymphedema model. In this model, a circumferential lesion is made at the base of the tail and the deep lymphatics are severed without damage to the systemic vessels. Intraperitoneal administration of 9-cisRA postoperatively reduced lymphedema, as assessed by tail diameter, and increased lymphatic capillary density, as assessed by immunohistochemistry. These findings were reproduced in a second strain of mice by a second set of investigators. So in conclusion, in 4 preclinical models, 9-cisRA enhanced angiogenesis. These animal data were compelling, and it appears that 9-cisRA, at the doses administered and over the time course of observation, had a lymphangiogenic effect.

**Time for a Trial of 9-cisRA in Lymphedema?**

I think not. A concern with any lymphangiogenic agent is the fact that for most patients in the United States, cancer (or its attendant therapy) is the cause of the lymphatic obstruction. Because the lymphatics play an important role in metastasis, a lymphangiogenic agent in these patients would need to be used cautiously, with its administration limited in time and space.

There remains a concern about what effect the agent has on the systemic vasculature. Although 9-cisRA did not induce proliferation of human systemic (dermal) endothelial cells and there was no evidence of local angiogenesis when 9-cisRA was implanted in the mouse cornea, these studies of angiogenesis were not comprehensive, nor did the investigators examine the effect of the agent on vasculogenesis (ie, mobilization of circulating endothelial progenitor cells). These data are needed because it would not be useful to expand the systemic microvasculature and to increase blood flow in the lymphedematous limb.

Furthermore, in some patients, any hydrodynamic solution for the swollen extremity will be insufficient. Although lymphedema begins as a lymphatic obstruction causing protein and fluid overload in the extremity, with chronicity of the condition, there may be dramatic tissue changes (not fully replicated in the mouse model). In this common progression of lymphedema, most of the dermal expansion is not due to fluid but rather is secondary to adipose tissue septated by bands of connective tissue with inflammatory infiltrate. This raises the question of what the effects of 9-cis RA might be on the process of lipogenesis, fibrosis, and inflammation that occurs with established lymphedema, given that 9-cisRA activates a signaling pathway (ie, FGF) known to stimulate the growth of a diversity of cells.

**What is the Future for Lymphedema Therapy?**

Nevertheless, lymphangiogenic therapies may have a role. In a mouse model of inherited limb edema based on mutations in VEGFR-3, therapeutic overexpression of VEGF-C using a viral vector induces lymphangiogenesis and improvement in lymphedema. Similarly, in a rodent model of surgically induced lymphatic obstruction, the exogenous administration of human recombinant VEGF-C restores lymphatic flow.

Direct microsurgical anastomotic procedures have been used to restore lymphatic flow. Lymphovenous anastomoses can be made between lymphatic vessels distal to an obstruction in nearby small veins. This procedure allows lymph from the obstructed region to flow directly into the venous system. Normal autogenous lymphatic vessels have been used to bypass lymphatic obstruction. However, the long-term patency of these microsurgical approaches is minimal. It is possible that better results may be achieved with autologous lymph node implantation, particularly if combined with lymphangiogenic therapy.

**In the Meantime, What Is to Be Done to Treat These Patients?**

The investigators state that “Lymphedema … presents a considerable physical and social burden because currently no treatment is available.” Perhaps the authors were referring to pharmacotherapy, which is certainly limited. Diuretics, although widely prescribed for this chronic, edematous condition, are rarely useful and may in fact be deleterious. Topical antifungals are useful because mycotic infections occur frequently, and antibiotics (particularly those directed at staphylococcus and streptococcus) are invaluable for the acute cutaneous infections to which these limbs are susceptible. However, chronic suppressive antibiotics have not been proven to prevent recurrences. Oral benzopyrone has its advocates, but a focused review of the available data concluded that the data were insufficient to support a recommendation.

On the other hand, physiotherapy for lymphedema can be very beneficial. In particular, complex decongestive physiotherapy, which includes specialized massage (manual lymphatic drainage) and multilayered bandaging, substantially reduces the girth of the extremity. Previously, we have shown that a reduction in limb volume of 40% can be achieved. These techniques can be combined with intermittent sequential pneumatic compression, which may augment the benefit. In addition, care should be taken to keep the skin well hydrated with moisturizing creams to avoid fissuring, which
would be a source for infection. Exercise should be encouraged as long as the limb girth is maintained with a compressive garment. Water sports are particularly good for patients with lymphedema because the limb is supported by the hydrostatic pressure of the water.

For patients with the chronic form of lymphedema characterized by adipogenesis, liposuction appears to be very useful. This surgical intervention is superior to the old reduction surgeries that could leave the patient with a painful, scarred limb ravaged by recurrent infections. Physiotherapy should be continued to maintain the surgical gain.

In conclusion, much can be done to ameliorate lymphedema. More definitive therapies, based on a better understanding of lymphatic development and lymphangiogenesis, are under investigation, as well as the pathological processes that contribute to and follow from lymphatic obstruction.

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References

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