

LIMB VOLUME REDUCTION AFTER PHYSICAL TREATMENT BY COMPRESSION AND/OR MASSAGE IN A RODENT MODEL OF PERIPHERAL LYMPHEDEMA

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ABSTRACT

Lack of a standardized experimental counterpart of peripheral lymphedema (LE) in a small animal has hampered research into treatment of this debilitating condition. We recently refined a rodent model consisting of radical unilateral lymphatic/nodal groin excision in conjunction with a circumferential integumental gap, followed by regional irradiation of the groin to reproduce stable unilateral hindlimb LE (1). In the current study, Wistar-Fuzzy rats with established right hindlimb LE, were subdivided into five groups and subjected to one of the following daily physical regimens over a 5-day period: pneumatic compression pumping at 30 torr (PCP); low-stretch multi-layered compressive bandaging using Coban (CB); manual lymphedema drainage (MLD) or a light massage consisting of stationary circular motions using the fingertips; combined physiotherapy (CPT consisting of MLD + CB); and a no treatment or control group (CTRL). Hindlimb and LE volumes were serially measured before and after treatment.

Whereas CTRL showed progressive worsening of hindlimb swelling, PCP, CB, CPT and MLD each produced similar and substantial edema reduction over the 5 day interval. PCP, CB and CPT induced vacillating edema reduction which, however,

exceeded rebound swelling on a daily basis. MLD, on the other hand, showed a steady gradual daily decline in LE volume.

Secondary lymphedema is a common long-term complication of regional lymphadenectomy and/or irradiation in the treatment of cancer. The accumulation of edema or interstitial fluid is a direct result of the imbalance between net microvascular filtrate (lymph formation) on the one hand, and the return of lymph to the bloodstream (lymph absorption) on the other hand. Impaired lymph return or low output failure of the lymph circulation is responsible for the accumulation of protein-rich interstitial fluid or what is termed lymphedema. Whereas the pathophysiologic, biochemical and molecular events that are responsible for scarring, fat deposition, and acanthosis in lymph stasis are still unclear, a wide variety of physical methods have been advocated to treat peripheral lymphedema. The basic mechanisms underlying these therapies and their relative efficacy have not been rigorously examined, thereby hampering progress in understanding and treatment of this life-long disabling condition. Experimental counterparts of peripheral lymphedema previously developed in large animals are costly, difficult to reproduce consistently, and have not been used systematically to study treatment (2-4).



Fig. 1. Photographs illustrating techniques of A) Compression Bandaging using a soft under layer of *Conform* and a low stretch outer layer of *Coban*, B) Manual Lymph Drainage of the jugular region, C) Pneumatic Compression Pumping using a size one neonatal blood pressure cuff over the foot and ankle and a size 2 cuff as a second layer that continues up to the mid-thigh over a layer of *Conform*.

We previously refined an inexpensive, reproducible rodent model of peripheral lymphedema, which simulates secondary lymphedema as seen in patients (1,5) This hindlimb preparation consists of radical lymphatic/nodal groin excision combined with regional irradiation. In the current study, we miniaturized and standardized physical treatment modalities advocated for peripheral lymphedema. These included manual lymphatic drainage (decongestion), low-stretch multilayered compressive bandaging alone or together with massage as “combined physiotherapy,” and a pneumatic compression pumping group. The effectiveness of each treatment modality was quantified and compared with each other and a no treatment or control group.

MATERIALS AND METHODS

Rat hindlimb LE development followed the protocol as previously described (1) and approved by University of Arizona Animal Care guidelines. Briefly, male Wistar-*fuzzy* rats, body weight ~ 250g, underwent deep lymphatic stripping in the right femoral and popliteal regions in conjunction with nodal excision and a superficial circumferential incision in the upper right thigh with skin

sutured to the underlying muscle layer leaving a ~ 5mm integumental gap. Approximately 2 days later 45Gy was delivered to a “medium” field encompassing the right groin. Approximately 21 days later, when rats had developed moderately severe but stable right hindlimb lymphedema, physical manipulations or control (CTRL) observations were carried out under ketamine anesthesia on randomized rat groups composed of cohorts whose LE volumes did not differ significantly (mean 48.2% increase in limb volume). MLD using a modified Vodder technique (6) was performed, but due to the small size of the rats, only stationary circular motions were applied to the axilla, groins, contralateral hindlimb and swollen limb. Bandage compression was accomplished using *Coban* (i.e., non-elastic bandage) snugly applied in conjunction with an under layer of *Conform* (soft under layer). This method produced evenly distributed, graduated and sustainable external compression. It was easily applied, removed and tolerated by the rats. CPT was carried out by first applying manual lymph drainage followed by the CB regimen. PCP was done using neonatal blood pressure cuffs on the foot to ankle of size one, layered by a size 2, which continued to the mid thigh. The cuffs

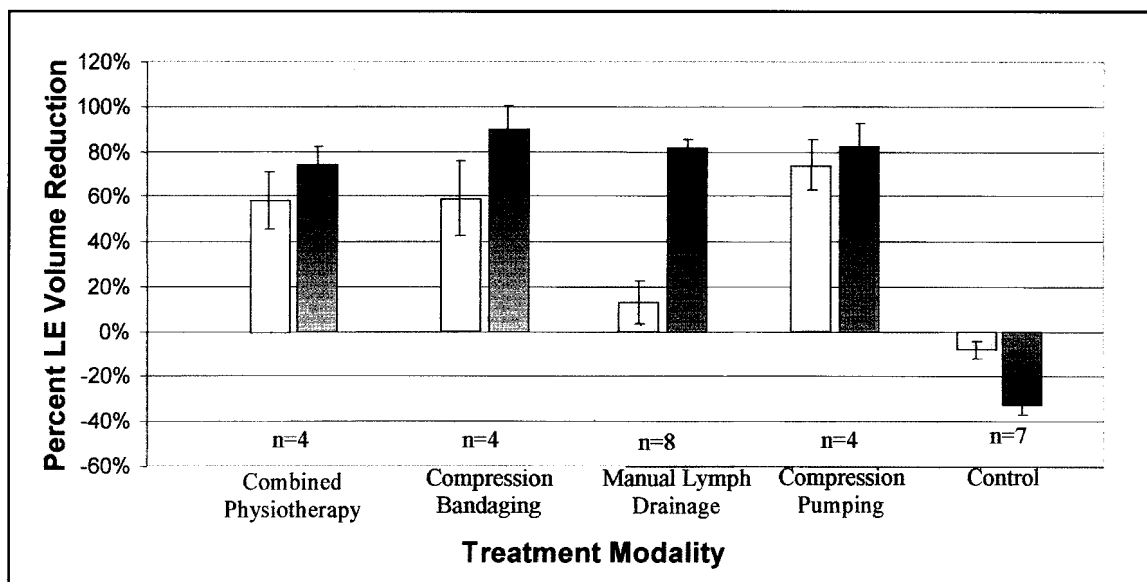


Fig. 2. Daily percent reduction in LE volume (light bars) and percent reduction in LE volume after 5 days of treatment (dark bars) by treatment group (mean \pm SEM). $p < .001$ when comparing Day 6 LE volumes of all experimental groups to the control group and $p < .001$ when comparing daily treatment group LE volume percent reductions to control and MLD, which did not differ significantly.

were placed over a layer of *Conform* and attached to a computerized unit to achieve 30 torr compression (Fig. 1). CB, PCP and CPT were applied for six hours on lightly anesthetized rats and repeated daily over a 5-day treatment period. Treatment effects were documented by incorporating serial standardized circumferential measurements on the experimental and control limbs using a modified truncated cone formula where limb volume (V) is calculated using the length of segment of the limb (h) and the serial circumferential measurements (c, C), according to the formula, $V = h(C^2 + Cc + c^2) / 12\pi$. Lymphedema volume (LEVol) was calculated by subtracting the contralateral hindlimb volume from the experimental limb volume. The percent increase (PI) was calculated by subtracting the contralateral limb volume (CLVol) from the experimental limb volume (ELVol) and dividing by the contralateral limb volume. The percent reduction (PR) was calculated by subtracting the initial lymphedema volume from the final lymphedema

volume and dividing by the initial lymphedema volume or: $LEVol = ELVol - CLVol$; $PI = 100 ((ELVol - CLVol) / CLVol)$; $PR = 100 ((LEVol_i - LEVol_f) / LEVol_i)$. The significance of treatment effects was analyzed using the student t-test.

RESULTS

Each treatment cohort and controls had substantial increases in hindlimb volume due to edema, and there were no significant differences in limb volumes among the groups at initiation of treatment [data not shown]. Each physical treatment modality tested not only prevented the progression of swelling, but also markedly reduced the edema ($p < 0.001$) over the 5 day treatment period when compared to the untreated control group (Fig. 2). Hindlimb edema worsened throughout the trial period in the control group (Fig. 3). There was no difference in total edema reduction over the 5 day treatment period among the four treatment

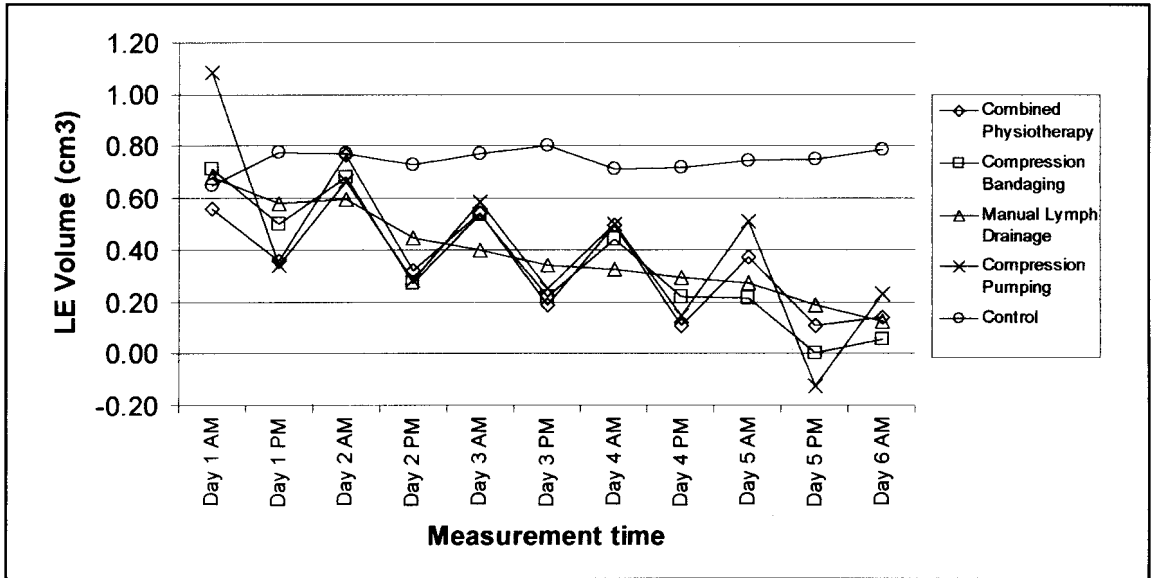


Fig. 3. Time course of daily regression of LE volume in each treatment group compared with control. Note daily rebound in all treatment groups except MLD although at 5 days reduction was similar.

modalities. The only notable difference among the treatment modalities was the daily “rebound effect” exhibited by CB, PCP, and CPT but not MLD, where edema reduction was more gradual but sustained (Fig. 3).

DISCUSSION

This study documents that standard physical treatment modalities commonly advocated for peripheral lymphedema can be miniaturized in a rodent model to allow for testing and comparisons of efficacy. Among the physical treatment techniques tested, manual lymph drainage (MLD) alone is as effective at reducing this subacute lymphedema as compression bandaging, pneumatic “pumping” or bandaging plus MLD. Moreover, edema reduction was uniform and progressive with MLD compared with the daily “rebound” phenomenon seen with the other methods. Nonetheless, each treatment method reduced LE volume similarly over a five-day period. The physiologic mechanism(s) behind the efficacy of these techniques, i.e., whether by decreasing net capillary filtration

(i.e., lymph formation) or increasing lymph return or both, remains to be clarified along with their efficacy in more chronic stages of lymphedema.

Although this study was restricted to examining edema reduction using standard physical treatment methods, this rodent model of the human condition also holds potential for investigating a wide variety of basic and clinical questions as well as evaluating preventive and therapeutic approaches to peripheral lymphedema. These include the response of lymphangiogenesis and lymphvasculogenesis during lymphatic obstruction, the role of putative growth factors such as VEGF-C and angiopoietin in the progression of lymphedema, and the value of measures to prevent or minimize lymphedema development.

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