The Use of Elastic Adhesive Tape to Promote Lymphatic Flow in the Rabbit Hind Leg

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Any method that deforms the skin of the extremities may increase lymphatic flow rate, and may be applied to treat peripheral lymphedema. This study was undertaken to investigate whether or not elastic adhesive tape with passive exercise can increase lymph flow in the rabbit hind leg by effective and periodic skin deformation. Cannulation into a pre-operated afferent lymphatic vessel in the lower left leg of 22 male New Zealand White rabbits was performed under a stereomicroscope. After stabilization, lymph was collected at rest or during passive exercise with an electric motor at 60 r.p.m. for 15 minutes and was then measured. Lymph flow rate was calculated and expressed as g/hour. Increase of lymph flow rate due to taping was significant only for passive exercise ($p=0.0317$). The lymph flow rate increased linearly as the area of tape was increased ($p=0.0011$), and lymph flow rates were significantly different according to site ($p=0.0017$). Tape on the anterior aspect of the ankle caused salient deformation and tended to increase the lymph flow rate more so than tape on the dorsum of the foot ($p=0.0831$). Taping with elastic adhesive tape in passive exercise increased the lymph flow rate in the rabbit hind leg by deforming the skin, which suggests a novel therapeutic method in cases of peripheral lymphedema.

Key Words: Bandages, lymphatic capillaries, physical therapy techniques, lymphedema, rabbits, continuous passive movement therapy, lymph flow, adhesive tapes, taping

INTRODUCTION

Lymph flow can be enhanced by activities such as walking, arterial pulsation, arteriolar vaso-motion, intestinal peristalsis, muscle movement, exercise, massage, or respiration, which are considered to cause tissue deformation. Without detectable contractile activity, the expansion and compression of the initial lymphatics, depending on the deformation of tissues in which they are embedded, can explain lymph formation and transport. Increased lymph flow rate, in this regard, was demonstrated quantitatively in the rabbit hind leg by passive exercise or periodic compression.

By inference, any method that deforms the skin of the extremities will also increase the lymphatic flow rate, and thus may be applied to the treatment of peripheral lymphedema. Current physical therapies involving compression bandages, manual lymph drainage, or exercise are somewhat related with this mechanism.

Adhesive tape, on the other hand, can cause excessive wrinkling - apparent tissue deformation - especially when it is placed on the skin near a joint and folds with flexure. Elastic adhesive tapes that are specially designed not to cause skin problems are widely used in sports. Exercise with this tape on one's skin may provide periodic expansion and compression of the initial lymphatics, suggesting a novel method of increasing lymph flow.

Thus, our study was designed to investigate the effect of elastic adhesive tape with or without passive exercise on lymphatic flow in the hind leg. To examine our hypothesis that this effect is
mechanical and related with skin deformation, we compared lymphatic flow rates according to the attached area and also the site where wrinkles could be formed differently.

MATERIALS AND METHODS

Animal preparation

Studies were carried out on 22 male New Zealand White rabbits (2.5 - 3.0 kg) anesthetized with a 5:1 mixture of ketamine chloride (5 mg/ml) and xylazine chloride (23.32 mg/ml), which was continuously infused through the ear vein at a rate of 1.4 - 1.6 ml/kg/hr using a syringe pump. Anesthesia was induced with the above mixture (0.2 ml/kg, iv) and maintained by infusion at 1.4 ml/kg/hr initially. Intermittent boluses were injected as needed during the experiment. If one or more boluses were needed in one hour, the rate was reset and maintained at 1.6 ml/kg/hr. Oral temperature was monitored and was maintained at 37.8 - 39.2°C using an electric heating pad.

The fur on the anterior aspect of the left hind leg and on the dorsum of the foot were cut using a clipper. To completely remove the remainder of the fur, a 4.48% thiglycolic acid cream (Neet®, Ildong, Seoul, Korea) was applied for 5 - 7 minutes, and the skin was then cleaned with a wet gauze.

Lymph cannulation and measurements

With a rabbit in the right decubitus position, lymph fluid was collected via a cannula (PE-10, Clay Adams) inserted under stereomicroscopic guidance into one of the lymphatic vessels in the lower left leg proximal to its entry into the popliteal node, as described previously. No contrast media were injected during the experiments, except during the preparatory phase of the study. Lymphatic vessels traveling almost parallel to the vein were found by engorgement by lightly massaging the dorsum of the foot.

The free tip of the cannula was placed 5 cm below the cannulation site to prevent lymph escape into another lymphatic pathway. Lymph fluid was collected in a 1.5-ml collector tube (Microtubes®, Sarstedt, Nümbrecht, Germany) through a hole made in the cap, which allowed only the passage of the cannula to minimize evaporation, which was negligible. The weight of collected lymph was measured using an electronic scale (d=0.1 mg, BP221S, Sartorius AG), and the lymph flow rate was determined by dividing the collected lymph weight by the collection time.

Passive exercise

The remaining fur on the left heel was fixed to a vise and a rod was connected to the base of the first toe. The opposite end of the rod was attached to an electric geared motor, thus periodically flexing and extending the ankle. The rod had an 8 cm oscillating diameter and the frequency of movement could be adjusted from 20 to 60 r.p.m. by means of a variable electrical resistance.

Experimental protocols

All experiments were carried out after a 15-min stabilizing period at 20 r.p.m. of passive movement after lymph cannulation. The passive movement was then stopped to change the tape for 30 seconds. To apply the tape on the skin, we rubbed the skin lightly several times. To remove the tape, we rolled it off from a corner and pushed the skin lightly in the opposite direction at the same time until the tape had been removed. The application and removal of tape was thought to produce the same effect on the lymphatics as light rubbing or light massaging. Thus, the light massaging was done before the very first lymph collection without tape to maintain the consistency of procedures throughout the experiment. To minimize the effect of this manipulation, we inserted an additional 5-min stabilizing period at 20 r.p.m. before every lymph collection. To offset the effect of the time required for animal preparation after anesthesia, the tape sequence of each protocol was repeated in reverse order. Lymph was weighted three times after collecting for a 15 minute period and the median weight was taken. Average lymph flow rate was calculated from this data.

Protocol 1: Lymph flow rate without passive exercise (N=4 animals)

After stabilization, lymph was collected with
and without elastic adhesive tape (9.0 cm × 2.4 cm; Kinesio Tex®, Kinesio, Tokyo, Japan). The tape was applied to the leg after being removed from its backing paper. Application was started at around the metatarsophalangeal (MP) joints on the dorsum of the left foot to the anterior aspect of the lower part of the leg while the ankle was extended.

For two rabbits the experiment sequence was: 15-min stabilization followed by 30-sec simulation, 5-min stabilization, 15-min collection without tape, 30-sec applying tape, 5-min stabilization, 15-min collection with tape, 30-sec changing tapes, 5-min stabilization, 15-min collection with tape, 30-sec taking off the tape, 5-min stabilization, and 15-min collection with tape. For the other two rabbits, lymph was first collected with tape. The measurements taken during the above two time protocols were similar and results were combined.

Protocol 2: Lymph flow rate with passive exercise (n=4 animals)
After stabilization, we suspended collection during passive exercise for 15 minutes at 60 r.p.m. to ensure similar conditions for the first collection. Simulation or taping for 30 seconds and stabilization for 5 minutes followed. The rest of the sequence of this experiment was the same as that detailed in Protocol 1, except that the lymph was collected during passive exercise at 60 r.p.m.

Protocol 3: Lymph flow rate according to the area of tape with passive exercise (n=7)
After stabilization and suspension as in Protocol 2, lymph was collected and measured for the following elastic adhesive tapes: 1/3 width; 9.0 cm × 0.8 cm, 2/3 width; 9.0 cm × 1.6 cm, 3/3 width; 9.0 cm × 2.4 cm, and no tape in passive exercise at 60 r.p.m. The tapes were applied as described in Protocol 1, placing 1/3 width and 2/3 width tapes in the middle of the leg. Lymph collection was started without tape, followed by a 1/3 width tape, a 2/3 width tape, and a 3/3 width tape, and then this was repeated in reverse order.

Protocol 4: Lymph flow rate according to the tape site with passive exercise (n=7)
After stabilization and suspension, as described in Protocol 2, lymph was collected and measured at different elastic adhesive tape sites (upper, anterior aspect of the lower leg including ankle, 4.5 cm × 2.4 cm; lower, dorsum of foot, 4.5 cm × 2.4 cm; whole, upper plus lower, 9.0 cm × 2.4 cm, and no tape) during passive exercise at 60 r.p.m. The tapes were applied as described in Protocol 1, except that the lower tape was attached only on the dorsum of the foot from the MP joints and the upper tape was attached from approximately 4.5 cm above the MP joints. The upper tape could produce excessive wrinkles around the anterior skin of the ankle in flexion, while the lower one could not. Lymph was collected beginning with no tape, followed by the upper tape, the lower tape, and finally the whole tape, and then repeated in reverse order.

Statistical analysis
All results are expressed as means ± standard deviation in tables and as means ± standard error in Fig. 1 and 2. The paired t-test was used to compare the lymph flow rates with and without tape with passive exercise, and also with no exercise. Associations between lymph flow rate and attached site or area of tape were evaluated by repeated measured analysis of variance followed by the Bonferroni method. First-degree linearity was also tested to investigate a possible linear association between area and lymph flow rate.

Differences between groups were considered significant at p<0.05. Statistical analyses were carried out using SAS for Windows version 8.0.

RESULTS
While there was no significant difference in the lymph flow rates with and without tape in the absence of exercise, taping during passive exercise exhibited significant rate increases (p=0.0317, Table 1). During passive exercise, the mean profiles of repeated measurements of the lymph flow rate were significantly different according to the area (p=0.0001, Fig. 1), and increased as the area of the attached tape increased, as indicated by a linear relationship (p=0.0011, Fig. 1).

The mean profiles of repeated measurements of
the lymph flow rate were also significantly different according to the site during passive exercise ($p=0.0017$). While the differences between the lower tape and no tape were not significant, differences between the upper tape and no tape were marginally significant ($p=0.0594$, Table 2). Differences between the lymph flow rates of the upper and lower tape was also marginally significant ($p=0.0831$), by paired t-test (Fig. 2).

When the ankle was flexed with the tape in position, wrinkles were apparent, but were not distinct without tape (Figs. 3A, 3B, 3C, and 3D; note the differences between the lengths of the marks to the anterior margin of the skin). Skin around the anterior aspect of the ankle, where the upper tape was attached, was deformed more so than skin of the dorsum of the foot, where the lower tape was applied when the ankle was flexed with the tape on (Fig. 3E). Naturally, the tape itself cannot cause wrinkling without motion so the degree of deformation of taped skin appears almost as the same as untaped skin in a neutral still position.

**DISCUSSION**

Currently, most investigators consider that initial lymphatic function is passive in terms of absorbing tissue fluid. Many ultrastructural features of initial lymphatics in different organs and different mammals closely resemble each other. Because of incomplete attachment between neighboring cells, the endothelium of initial lymphatics may act as a microvalve along the walls of the initial lymphatics. These junctions can open during stretching of the lymphatics and during influx of interstitial fluid into the lumen, while anchoring filaments keep the endothelial cells tightly attached to the adjacent collagen network. Expansion of the initial lymphatics causes filling by percolation of the interstitial fluid across the open endothelial microvalves; whilst compression causes closure of the endothelial microvalves and outflow along the lumen of the miclymphatics, with eventual transport to collecting lymphatics. Reflow towards the initial lymphatics is prevented by bicuspid valves.

In this study, we hypothesized that degrees of compression and expansion could be enhanced by taping and thus deforming skin tissue more than without taping. Taping is simpler and requires less training than the application of a compression bandage. Moreover, if taping has a proven positive effect it could be used as a treatment method for peripheral lymphedema, which needs lifelong care and patient compliance.

Numerous studies have shown that limb motion enhances lymph flow. The increases in lymph flow rate due to physical activity in the present study agree with the findings of the majority of other studies (Table 1). The very low lymph flow rates with no movement also concur with the findings of others. The maintenance of lymph flow in a resting state can also be explained by rhythmic tissue motion like respiration or arterial pulsation.

Lymph flow rate was found to increase when the hind leg was exercised passively with taping by 24.4 - 37.5% (Table 2) versus without taping. Lymph flow can be increased by local hyperthermia. A local skin temperature when covered by tape may be elevated, thus increasing the microcirculation and lymph formation. However, when any deformation of the tissue was not expected, taping itself was not found to increase the lymph flow rate without passive movement (Table 1). The linear association between the

<table>
<thead>
<tr>
<th>Passive exercise</th>
<th>Lymph flow rate (g/hour)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No tape</td>
<td>Tape</td>
</tr>
<tr>
<td>Without (n=4)</td>
<td>0.0047 ± 0.0025</td>
<td>0.0045 ± 0.0028</td>
</tr>
<tr>
<td>With at 60 r.p.m. (n=4)</td>
<td>0.0447 ± 0.0151</td>
<td>0.0556 ± 0.0151</td>
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Values are mean ± standard deviation.

P-values are calculated by paired t-test.
Table 2. Lymph Flow Rate According to the Site of Tape in Passive Exercise (n=7)

<table>
<thead>
<tr>
<th>Site of tape</th>
<th>Lymph flow rate (g/hour)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>0.0416 ± 0.0225</td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td>0.0488 ± 0.0306</td>
<td>0.2244</td>
</tr>
<tr>
<td>Upper</td>
<td>0.0537 ± 0.0298</td>
<td>0.0594</td>
</tr>
<tr>
<td>Whole (upper+lower)</td>
<td>0.0572 ± 0.0301</td>
<td>0.0270</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation.
P-values are calculated by repeated measured analysis of variance and corrected by Bonferroni method comparing each value with no tape.

Fig. 1. Lymph flow rates were calculated for different tape areas for passive exercise at 60 rpm, and a significant difference (p=0.0001) was observed by repeated measured analysis of variance. Linearity was also tested and exhibited significance (p=0.0011). The values shown represent means and standard errors (vertical bar).

Fig. 2. Lymph flow rates with upper and lower positioned tapes were compared by paired t-test, and found to be marginally different (p=0.0831). The values shown represent means and standard errors (vertical bar).

Lymph flow rate and the area of the tape in the present study (Fig. 1), is very similar to that found in a previous study, in which lymph flow rates generated by the massage of two neighbouring points were almost linearly additive. This phenomenon can be largely explained mechanically.

If two different objects are closely attached to each other, like bimetal in a thermostat, they will bend, or deform themselves when their lengths change differently during expansion or shrinkage. Likewise, when tightly attached elastic adhesive tape is stressed by movement, it adjusts its length to that of the skin only to an extent, and thus deforms the skin (Figs. 3B and 3D). It remains a possibility that non-elastic adhesive tape might be more effective than an elastic tape since it is not as skin compatible. This aspect needs to be examined in future experiments.

Changes in the lengths of tissues are extreme around a flexed or extended joint. Major wrinkles are formed near or around the joint when tape is applied (Fig. 3E). Though the difference between the lymph flow rates of two sites was not found to be significant in our study, it could be significant if the number of rabbits increases. This apparent skin tissue deformation seems to provide a possible explanation of why lymph flow appears to be faster when tape is applied around the ankle as opposed to the dorsum of the foot (Table 2, Fig. 2). However, there is more to consider. Since interstitial fluid is largely absorbed into the initial lymphatics, with only limited absorption into collecting lymphatics, external compression functions best when it is applied to the initial lymphatics.
rather than to the collecting lymphatics. In addition, initial lymphatics in skin lie just below the dermis. In the majority of studies, a superficial subpapillary layer of initial lymphatics is described. Wrinkling appears not only to deform the superficial skin layer, but also the deep subcutaneous tissue, where initial lymphatics are believed to be sparse. Only a slight pressure drop (0.1 cmH₂O) from the interstitium is enough for lymph formation in initial lymphatics. Lymph flow increases 12-50 fold when the interstitial fluid pressure increases from its control level of about -6 mmHg up to 0 mmHg. Above or below this level, no further increase in lymph flow is expected. Thus, too much applied pressure or deformation may be unnecessary or even harmful.

Fig. 3. A) The distance from the marked point on the lateral malleolus to the anterior margin of the skin was about 8 mm when the ankle was extended without tape. B) The distance from the marked point on the lateral malleolus to the anterior margin of the skin was about 8 mm when the ankle was extended with tape. C) The distance from the marked point on the lateral malleolus to the anterior margin of the skin was about 9 mm when the ankle was flexed without tape. D) The distance from the marked point on the lateral malleolus to the anterior margin of the skin was about 12 mm when the ankle was flexed with tape. E) The skin around the anterior aspect of the ankle was more deformed than that of the dorsum.
in the long run in terms of increasing lymph flow. It is, therefore, difficult to explain increased lymph flow exclusively by this static pressure, unless taping around the ankle happens to apply an aforementioned range of pressure. One thing peculiar about a wrinkle is that it is not only depressing, it also elevates the skin, like a valley and a mountain (Fig. 3D and 3E). This must have some positive effect on the formation of lymph by opening microvalves in the initial lymphatics. Therefore, the generation of appropriate dynamic pressure variation and sufficient periodic compression and decompression of superficial and deep initial lymphatics sounds like a plausible explanation for increasing lymph flow around a joint.

On the other hand, faster lymph flow when tape is applied around the ankle may be explained by the anatomy of the lymphatic system of the rabbit hind limb. It appears that there are at least two different anatomic regions of the lymphatic system in the foot and ankle. In our study, the system we used drains the ventral, ventromedial and ventrolateral aspects of the foot into several afferent lymphatic vessels, which finally enters the popliteal node. It is not known yet if each one of these vessels drains lymph evenly from this area. Accordingly, there is a chance that cannulation of one of these vessels may drain more lymph from the ankle than the dorsum of the foot.

Though the effect of taping with exercise is shown by our study, there remains some problem concerning the tape used as a novel treatment for lymphedema. Both efficacy and adverse reactions should be evaluated for various types of tape, for example, some adhesives might be allergic to some patients. Friction, depending on the elasticity of the tape could irritate the skin. And we suggest further study involving the measurement of intra-lymphatic pressure should also be carried out in the future to examine if lymph flow can overcome gravity or obstructive pressure.

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