Exploring the Effect of Osteopathic Medicine on the Lymphatic System

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• “We strike at the source of life and death when we go to the lymphatics” - A.T. Still
The lymphatic system

- The lymphatic system preserves tissue health, aids in the absorption of gastrointestinal lipids, and promotes immune surveillance.
- Failure of the lymphatic system has been implicated in the pathogenesis of a variety of diseases including cardiovascular disease, inflammation, and edema.
The lymphatic vessels

- Lymphatic vessels drain fluid from the tissue and continuously provide information about the tissue to the immune system.
- Lymph vessels transport microbial antigens, tissue antigens, toxins, cytokines, chemokines, apoptotic cells and immune cells from tissue to the regional lymph nodes.
Clinical significance

• Diseases that impair lymph flow, such as infection and lymphedema, hinder lymph recirculation.

• Interventions that improve lymph flow may relieve edema and treat infection by enhancing the circulation of immune cells, inflammatory mediators, and pharmaceuticals.
Therapies that enhance lymphatic circulation

- Physical/manual therapies have been shown to increase lymphatic flow in humans and animals.
  - Exercise (aerobic and resistance)
  - Passive limb rotation
  - Massage/lymphatic drainage
  - Pneumatic compression devices
  - Osteopathic manipulative treatments (OMT)
Osteopathic techniques that were designed to enhance lymph circulation

- Myofascial release, traction, and release of diaphragms
  - remove restrictions to lymphatic vessels
- Lymphatic pump techniques (LPT) enhance flow of lymph through the vessels
  - Thoracic, abdominal, splenic, liver, and pedal pumps
Anecdotal evidence for the use of OMT to enhance immunity and treat pneumonia

- OMT increased blood leukocyte numbers (Castlio and Ferris-Swift 1934, Noll et al. 2008, Mesina et al. 1998)
- OMT improved sputum production and shortened duration of cough (Allen et al. 1967).
- OMT enhanced vaccine specific antibodies (Measel 1982, Jackson et al. 1998)
- OMT decreased the length of hospital stay and the need for antibiotics in elderly patients with pneumonia (Noll et al. 2000, Noll et al. 2010).
- OMT induced early plasma cytokine release and mobilization of a population of blood dendritic cells (Walkowski et al. 2014)
- Collectively, these results suggest that OMT can enhance the immune system and protect against pneumonia.
Animal models used to study the effect of manual therapies on the lymphatic system

The effects of manually applied intermittent pulsation pressure to rat ventral thorax on lymph transport.

- A fluorescent probe was injected into the interstitial fluid space of anesthetized rats.
- Thoracic LPT significantly increased the rate of appearance of the fluorescent probe in blood compared to that observed in untreated control animals.
- Since the probe could not cross the vascular capillaries, these results indicated that **LPT enhanced the uptake of the probe by the lymphatic system and its transport from tissue to blood.**

Increased lymphatic flow in the thoracic duct during manipulative intervention.

- Dogs were surgically instrumented to measure thoracic duct lymph flow.
- After recovery from surgery, the dogs received thoracic lymph pump, abdominal lymph pump, or treadmill exercise.
  - All three interventions significantly increased thoracic duct flow compared to pretreatment.
  - The greatest increases were seen during abdominal pump and exercise.

Does LPT alter the composition of lymph?

- By stimulating lymph flow, LPT may mobilize immune cells and inflammatory mediators into lymphatic circulation.
LPT increases the lymphatic flux of leukocytes in thoracic duct lymph

Thoracic duct lymph was collected 1) pre-LPT, 2) during 4 min LPT, and 3) during 10 min post-LPT. Data are means x 10^6 total leukocytes/minute ± SE or mean arterial blood pressure ± SE from 6 animals. *Greater than Pre-LPT and Post-LPT (P < 0.01).
LPT increases leukocyte flux in thoracic duct lymph

<table>
<thead>
<tr>
<th></th>
<th>Pre-LPT</th>
<th>LPT</th>
<th>Post-LPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>0.27 ± 0.12</td>
<td>3.67 ± 0.96**</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.34 ± 0.14</td>
<td>4.24 ± 1.18*</td>
<td>0.36 ± 0.10</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>10.32 ± 4.53</td>
<td>81.1 ± 22.2**</td>
<td>7.30 ± 2.30</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>3.25 ± 0.62</td>
<td>43.7 ± 5.57**</td>
<td>5.10 ± 1.90</td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>1.24 ± 0.37</td>
<td>16.3 ± 4.12**</td>
<td>2.23 ± 0.76</td>
</tr>
<tr>
<td>IgA+ B cells</td>
<td>0.65 ± 0.18</td>
<td>9.02 ± 0.86**</td>
<td>0.60 ± 0.21</td>
</tr>
<tr>
<td>IgG+ B cells</td>
<td>1.06 ± 0.21</td>
<td>13.4 ± 4.81*</td>
<td>0.78 ± 0.18</td>
</tr>
</tbody>
</table>

Thoracic duct lymph was collected 1) pre-LPT, 2) during 4 min LPT, and 3) during 10 min post-LPT. Data are means x 10^6 total leukocytes/minute ± SE from 6 animals. *Greater than Pre-LPT and Post-LPT (P < 0.01).
LPT increases the lymphatic flux of cytokines in thoracic duct lymph

Thoracic duct lymph was collected 1) pre-LPT, 2) during 4 min LPT, and 3) during 10 min post-LPT. Data are means × 10⁶ total leukocytes/minute ± SE from 6 animals. Data are means±SE (n = 6). *Greater than respective pre-LPT and post-LPT (P<0.05). **Greater than respective pre-LPT and post-LPT values (P<0.01). ***Greater than respective pre-LPT and post-LPT values (P<0.001).

Does LPT stimulate mesenteric lymphatics?

- LPT compresses tissues in the abdominal area, which may facilitate the release of immune cells and inflammatory into thoracic duct lymph.
LPT increases the lymphatic flux of leukocytes in intestinal duct lymph

Intestinal duct lymph was collected 1) pre-LPT, 2) during 4 min LPT, and 3) during 10 min post-LPT. Data are means x 10^6 total leukocytes/minute ± SE or mean arterial blood pressure ± SE from 6 animals. *Greater than Pre-LPT and Post-LPT (P < 0.01).

LPT increases leukocyte flux in intestinal duct lymph

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Baseline (x 10^6/minute ± SE)</th>
<th>LPT (x 10^6/minute ± SE)</th>
<th>Recovery (x 10^6/minute ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>0.12 ± 0.03</td>
<td>1.79 ± 0.47**</td>
<td>0.22 ± 0.07</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.10 ± 0.02</td>
<td>1.10 ± 0.30**</td>
<td>0.29 ± 0.13</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.85 ± 0.23</td>
<td>13.1 ± 4.43**</td>
<td>1.90 ± 0.98</td>
</tr>
<tr>
<td>CD4^+ T cells</td>
<td>1.36 ± 0.36</td>
<td>9.00 ± 3.47**</td>
<td>1.30 ± 0.50</td>
</tr>
<tr>
<td>CD8^+ T cells</td>
<td>0.27 ± 0.08</td>
<td>1.84 ± 0.55**</td>
<td>0.29 ± 0.10</td>
</tr>
<tr>
<td>IgA^+ B cells</td>
<td>0.28 ± 0.13</td>
<td>4.10 ± 3.42*</td>
<td>0.14 ± 0.05</td>
</tr>
<tr>
<td>IgG^+ B cells</td>
<td>0.14 ± 0.06</td>
<td>2.00 ± 1.29*</td>
<td>0.45 ± 0.04</td>
</tr>
</tbody>
</table>

Intestinal duct lymph was collected 1) pre-LPT, 2) during 4 min LPT, and 3) during 10 min post-LPT. Data are means x 10^6 total leukocytes/minute ± SE from 6 animals. *Greater than Pre-LPT and Post-LPT (P < 0.01).
LPT increases the lymphatic flux of cytokines in mesenteric duct lymph

Intestinal duct lymph was collected 1) pre-LPT, 2) during 4 min LPT, and 3) during 10 min post-LPT. Data are means x 10^6 total leukocytes/minute ± SE from 6 animals. Data are means+SE (n = 6). *Greater than respective pre-LPT and post-LPT (P<0.05). **Greater than respective pre-LPT and post-LPT values (P<0.01). ***Greater than respective pre-LPT and post-LPT values (P<0.001).

LPT mobilizes lymphocytes from mesenteric lymph nodes

The mesenteric lymph nodes were fluorescently labeled \textit{in situ}. Thoracic duct lymph was collected 1) pre-LPT, 2) during 4 min LPT, and 3) during 10 min post-LPT. Data are means $\times 10^6$ total leukocytes/minute $\pm$ SE from 6 animals. *Greater than Pre-LPT and Post-LPT ($P < 0.01$).

Four minutes of LPT

• 1) enhanced lymph flow,
• 2) increased the concentration of leukocyte in lymph
• 3) mobilized inflammatory mediators into circulation, and
• 4) stimulated mesenteric immune cells to enter lymph circulation.
• In a **HEALTHY** animal
How does LPT protect against disease?

- Clinical studies support the use of OMT, including LPT, for the treatment of pneumonia.
- The mechanisms of protection are unknown.
- Small animal models allow researchers to study of the mechanisms of action of OMT during disease.
LPT increases lymphatic leukocyte flux in rats

Table 1. Lymphatic Pump Treatment Increases Lymphocyte Flux in Cisterna Chyli Lymph.

<table>
<thead>
<tr>
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<th>Pre-LPT</th>
<th>LPT</th>
<th>Post-LPT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymphocyte Flux (x 10^6 cells/min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total lymphocytes(^a)</td>
<td>0.63 ± 0.20</td>
<td>5.90 ± 0.99**</td>
<td>0.52 ± 0.13</td>
</tr>
<tr>
<td>CD4(^+) T cells(^b)</td>
<td>0.37 ± 0.17</td>
<td>3.60 ± 0.54**</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>CD8(^+) T cells</td>
<td>0.11 ± 0.05</td>
<td>1.00 ± 0.28**</td>
<td>0.09 ± 0.06</td>
</tr>
<tr>
<td>B cells</td>
<td>0.14 ± 0.01</td>
<td>0.70 ± 0.50*</td>
<td>0.08 ± 0.02</td>
</tr>
</tbody>
</table>

Lymph was collected during four min pre-LPT, four min LPT and 10 min post-LPT. Values are means ± SE or means x 10^6 lymphocytes/min from 10 experiments. \(^a\) Concentration of total lymphocytes. \(^b\) Concentration of total lymphocytes that were T or B cells. ** P<0.01, compared to pre-LPT and post-LPT. *P<0.05, compared to pre-LPT and post-LPT.
LPT releases intestinal lymphocytes into the lymph of rats

**FIG. 2.** Cisterna chyli lymph was collected during 1) pre-LPT, 2) 4 min LPT, and 3) 4 min post-LPT. Data are means × 10^6 total of α4β7 positive lymphocytes/min ± SE from 10 animals. *Greater than pre-LPT and post-LPT (p < 0.05).
Does LPT protect against pneumonia in a rat model?

- OMT decreased the length of hospital stay and the need for antibiotics in elderly patients with pneumonia (Noll et al. 2000, Noll et al. 2010).
- LPT might redistribute lymph-borne factors and pharmaceuticals to the lung and provide additional protection against pneumonia.
Does LPT protect against pneumonia?

On day 0 rats were nasally infected with $5 \times 10^7$ S. pneumoniae colony forming units (CFU). Treatment was applied once a day at 24, 48 and 72 hours post-infection. Lungs and blood were collected at 48, 72 and 96 hours post-infection.
LPT reduces bacteria in the lungs of rats

Data were analyzed by analysis of variance (ANOVA) followed by a Tukey multiple comparisons post test. *Differences among mean values with P<0.05 were considered statistically significant. Data are means ± standard error (SE).

Does LPT enhance the effect of antibiotics?

LPT acts as an adjunctive therapy during pneumonia

Table 1
LPT plus Levofloxacin Protect against S. pneumoniae

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent disease free</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + PBS</td>
<td>0</td>
</tr>
<tr>
<td>Sham + PBS</td>
<td>0</td>
</tr>
<tr>
<td>LPT + PBS</td>
<td>13</td>
</tr>
<tr>
<td>Control + levofloxacin</td>
<td>25</td>
</tr>
<tr>
<td>Sham + levofloxacin</td>
<td>38</td>
</tr>
<tr>
<td>LPT + levofloxacin</td>
<td>63*</td>
</tr>
</tbody>
</table>

* Exceeded the upper limit by Analysis of Means for proportions
Conclusions

• LPT increased the concentration of leukocytes and the flux of inflammatory mediators in lymph.
• LPT stimulated the release of leukocytes from the gastrointestinal lymphoid tissues
• LPT acts as an adjunctive therapy to antibiotics during the treatment of pneumonia
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