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THE EFFECTS OF MANUALLY APPLIED INTERMITTENT PULSATION PRESSURE TO RAT VENTRAL THORAX ON LYMPH TRANSPORT

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ABSTRACT

The present study evaluated the effects of tissue massage on a part of the body remote from the region of lymph uptake into the initial lymphatics. Lymph uptake was assessed with a fluorescent probe placed in a potential space of the lower extremity of anesthetized female Sprague-Dawley rats. Tail blood was assayed at intervals over 15 hours for fluorescence. A total of 63 animals were utilized (treatment = 32 and control = 31). The manipulated group received lymph flow enhancing treatment (LFET) five minutes per rat per hour until they were aroused. The control group were left lying prone in cages until a blood sample was taken. The LFET procedure was bilateral finger pressure applied to the lower ribs of a supine rat followed immediately by a light tap to the sternum. These maneuvers were repeated for 5 minutes. The rate of appearance of fluorescent probe was greater during the first nine hours of the experiment in the treatment group than in the controls but not at hours 12 and 15. This study demonstrates that mechanical pressure to body regions physically distant from the location of lymph formation enhances lymph uptake.

Osteopathic physicians have long advocated that tissue manipulation increases interstitial fluid movement into the lymphatics. This conviction was based on clinical observations that tissue manipulation improves pathological conditions involving lymph stasis such as an ankle sprain and the accompanying edema. Before the advent of modern infectious disease pharmacotherapy, tissue manipulation designed to promote lymph flow was commonly used to treat influenza. Indeed, Smith reported that during the influenza pandemic of 1917, patients that received tissue manipulation had a mortality rate of 0.25% as compared with 5% that did not receive such manipulation (1).

Lymphatic transport serves to minimize interstitial fluid accumulation. Several mechanisms regulating lymph propulsion have been elucidated including adjacent arterial pulsation (2), skeletal muscle contraction (3), walking (4), exercise (5), respiratory excursion (6), passive joint movement (7), and massage (8-10). Lymphatic collectors which contain smooth muscle also undergo periodic contraction and are sensitive to various mediators such as neural (11), humoral (12), and endothelial dependent factors (13,14).

Whereas lymph in the collecting lymphatics is propelled by endothelial smooth muscle and rhythmic contractions, it is less clear what determines movement of interstitial fluid into the initial lymphatics. The latter are devoid of smooth muscle, start abruptly in the interstitium, and are remote from the arterial-capillary-venous circulation.

Ikomi et al determined tissue fluid uptake into the initial lymphatics depended on tissue deformation or skin massage (9,10). In one such study (10), these researchers found that massaging two neighboring points on the skin produced lymph flow that was nearly linearly additive as compared to massaging each point individually.

The present study was designed to evaluate the effects of tissue massage on a part of the body remote from the region of lymph formation in the initial lymphatics. Massage was applied to the ventral thorax and lymph uptake was assessed by using a fluorescent probe that was previously inserted into a potential space of the hindlimb of a rat. The fluorescent probe circulated and blood was collected at intervals over 15 hours and quantitatively measured with a spectrofluorometer to determine whether remote tissue manipulation enhanced lymph flow.

METHODS

These experiments were reviewed and approved by the Institutional Animal Care and Utilization Committee of the University of New England. Female Sprague-Dawley rats, weighing between 150-250 grams, were used. Rats were allowed food and water *ad lib* and maintained on a 12/12 h light/dark cycle. They were housed in groups of 3-4 until used. After the experiments, all rats were euthanized with an overdose of carbon dioxide.

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Additional sodium pentobarbital (1-2 mg) was injected as needed during the experiment to ensure an acceptable level of anesthesia, as determined by lack of response to toe-pinch. Anesthesia was terminated after closure of the wound for introduction of the fluorescent probe. Baseline blood samples were taken from each rat (see protocol below) and a standardized volume of 100 ml containing 0.83 mg fluorescein labeled bovine albumin (fluorescein conjugate, Molecular Probes, Inc., Eugene, Oregon) was inserted through a 4 mm incision into a potential space in the right thigh between the two heads of the rectus femoris muscle. The wound was closed with surgical staples to the overlying skin.

Blood Sampling and Measurement of Fluorescein

Blood samples were taken at base-line pre-probe placement and then 2, 4, 6, 9, 12, and 15 hours post-probe placement. To assess the fluorescein concentration in the plasma, 0.2 ml of blood was collected from the nicked tip of the rat's tail and placed into a heparinized capillary tube. The blood was centrifuged for 5 minutes and 20 mmol of the serum was diluted with 0.7 cc of 0.14 M saline. The concentration of the fluorescein in the solution was assessed by a Jasco FP-770 spectrofluorometer. Excitation occurred at 490 nm and emission was recorded at 520 nm. The sensitivity was fixed at 100%. The peak heights, measured in millimeters, was the criterion variable used.

Lymph Flow Enhancing Treatment

The experimental group received lymph flow enhancing treatment (LFET) five minutes per rat per hour until they were aroused and would not permit manipulation; rats typically awakened in four hours. The control group were left lying prone in cages until a blood sample was taken. The LFET procedure consisted of placing the animal supine on a counter top and taking hold of the lateral aspect of the lower ribs with thumb and forefinger. Bilateral finger pressure was applied for approximately 0.5 second and then released. This was followed immediately by a light tap to the sternum. This procedure was repeated for 5 minutes. (The rationale for the sternal tap after each compression was to encourage inspiration.) The treatment group was left prone in cages after each LFET or blood draw.

Data Analysis

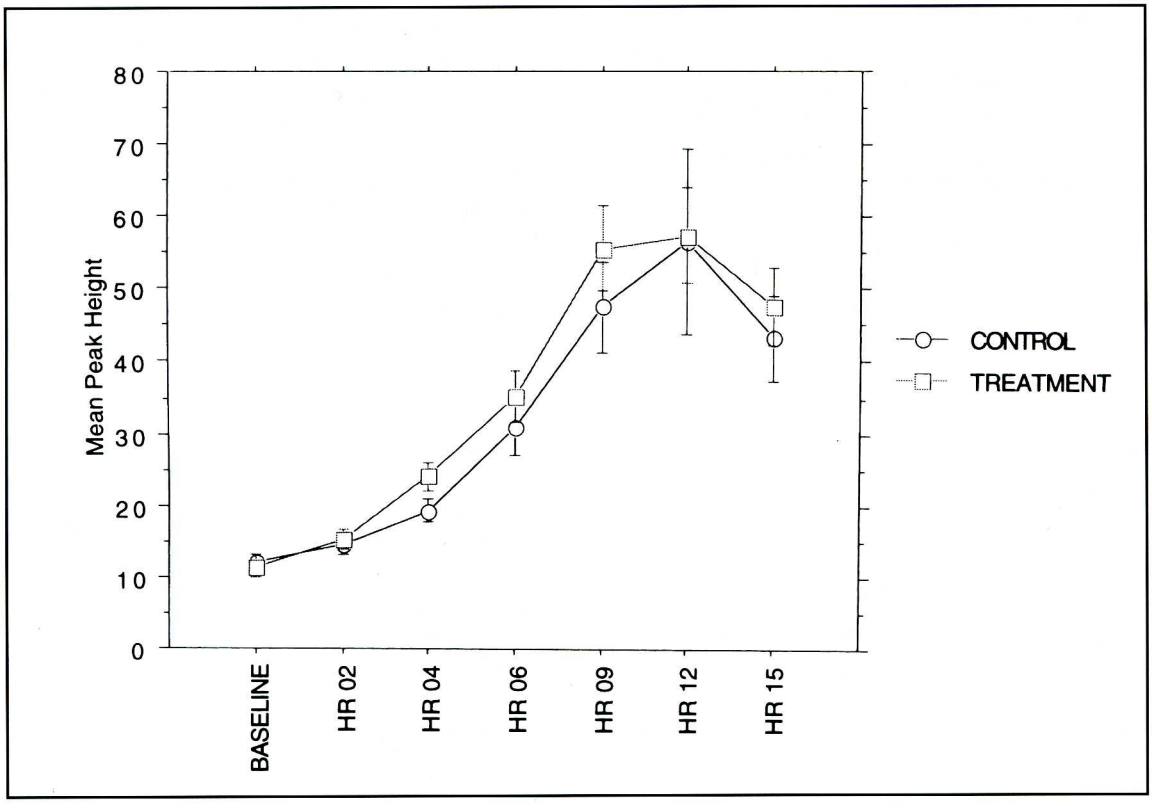

Fig. 1. Peak heights of albumin fluorescein complex measured over time. Vertical bars represent 95% confidence intervals.

The analysis of the data was conducted on JMP Statistics for the Macintosh (SAS, INC, North Carolina). A two factor repeated measures MANOVA (1 within, 1 between) analysis was utilized to determine if differences between the groups existed over time. The dependent variables measured were the peak heights obtained from the spectro-fluorometer. The independent variable was the animal group, treatment or control. A power analysis was conducted a priori. To obtain a power of 79%, a total of 55 animals were needed.

RESULTS

A total of 63 rats were utilized (treatment = 32 and control = 31). *Fig. 1* shows the average peak height for each group over the complete 15 hour experimental protocol. Note that the rate of appearance of fluorescent probe is greater in the first nine hours of the experiment in the treatment group than the controls. By inference this difference suggests that the rate of lymph uptake was greater for the manipulated group. At hours 12 and 15 the differences were indiscriminate. This finding was supported by the two factor repeated measures MANOVA. In the first nine hours there was a significant difference between the treatment and control groups ($p = 0.02$). There was also a significant time effect (within groups) ($p = 0.01$).

DISCUSSION

Fluid and proteins in the interstitium are transported back to the systemic circulation

through lymphatics. Collecting lymphatics but not initial lymphatics contain smooth muscle and through intrinsic contraction, propel lymph toward the bloodstream. The present results suggest that remote tissue manipulation enhances lymph uptake into the initial lymphatics and possibly increases lymph flow through the collecting lymphatics. Cannulation of the lymphatics to directly measure the change in the rate of flow with LFET may in the future validate this conclusion.

This study demonstrates that mechanical pressure to body regions remote from lymph formation enhances lymph absorption. In turn, this finding supports the hypothesis that lymph pump manipulation also increases the immune response, a theory that Smith advanced to explain the reduction in mortality of patients treated by osteopathic physicians during the 1917 influenza epidemic (1). As lymph is propelled through lymph vessels, the fluid filters through lymph nodes that contain lymphocytes pivotal for immunological responsiveness. Indeed, Ironson et al has proposed after a small study (N=29) that daily massage in men infected with acquired immunodeficiency virus (HIV+) increases bloodstream natural killer cells, natural killer cell cytotoxicity, soluble CD8, and the cytotoxic subset of CD8 cells (15).

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