LYMPHOLOGY
LYMPHATICS • LYMPH • LYMPHOCYTES • LYMPH NODES

PROGRESS IN LYMPHOLOGY XXIV

Proceedings of the 24th International Congress of Lymphology Rome, Italy September 16-20, 2013

Editor: Sandro Michelini

LYMPHOLOGY 47 (Suppl): 1-320, 2014 **ISSN 00247766**

SPECTROPHOTOMETRIC DETERMINATION OF DYE ACCUMULATION IN THE LYMPH NODES OF MICE: A COMPARATIVE STUDY BETWEEN LOCAL MASSAGE AND EXPOSURE TO MULTIDIRECTIONAL VIBRATIONS IN THE HORIZONTAL POSITION (ANDULLATION)

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ABSTRACT

Background

Whole body vibrations (WBV) are frequently applied by sportsmen, in well-being centres, and for medical conditions. The physiological effects of these vibrations have been studied extensively.

Objective

Analysing the short term effects of multidirectional vibrations delivered in a horizontal position (andullation) and local massage on the accumulation of dye in the lymph nodes of mice.

Methods

Whole body vibrations at 30 Hz in a horizontal position (andullation) during 5 minutes as well as local massage for 5 minutes are applied ensuing injections of 20 µl Evans blue dye (EBD) in the foot-pads of 2 randomized groups of mice (total N = 15 animals). Both groups are compared to a non-treated, randomized, but similarly injected control group (N = 10 mice). Following animal euthanasia, the EBD-stained popliteal and sacral lymph nodes are carefully resected and their contained blue dye extracted. The quantity of EBD (µg) is determined using a spectrophotometric technique with a wavelength of 620 nm.

Results

As a result of total body vibrations in the horizontal position the quantities of EBD in the popliteal lymph nodes were statistically higher compared to the control group: respectively 0.56 ± 0.26 and 0.14 ± 0.17 (mean and sd, p?0.05). The quantities of EBD also differed statistically from the recorded quantities following massage: respectively 0.56 ± 0.26 and 0.22 ± 0.16, mean and sd, p?0.05). The quantifications of EBD in sacral lymph nodes revealed strictly identical values.

Conclusion

The presented experiment in mice indicates that high quantities of EBD are accumulated in the lymph nodes following a short whole body exposure to multidirectional vibrations (30

Hz) in the horizontal position. The quantity of accumulated dye is higher than when local massage is performed at the dye-injection site without intervention.
The specific tracer accumulation in the lymph node is an important lymph function parameter and depends on the quality of lymph reabsorption and lymph flow.

Keywords

Andullation, Horizontal vibrations, Massage, Lymph nodes dye accumulation, Microcirculation, Evans Blue Dye, Spectrophotometric analysis

INTRODUCTION

Whole body vibrations delivered in a vertical position are applied by sportsmen as well as during well-being sessions. Significant physiological effects have been demonstrated on the human body (Prisby e. a., 2008). Positive effects are recorded in the medical field and particularly in elderly people being treated in geriatric rehabilitation services (Bautmans, e. a., 2005; Eismans, 2001). In 2007, a new technique of whole body vibration was introduced for medical applications. The innovative andullation technology combines infrared light with stochastic vibrations. The two biophysical treatment applications are delivered to an individual in the horizontal position on a massage mattress which is beneficial to the bedridden patient. Massage by mechanically induced vibrations in the recumbent position is of interest as a potential additional prevention or treatment in pathological conditions of both blood-vascular and lymphatic circulatory systems. The aim of this study is to investigate the effect on the lymphatic system of short exposure to multidirectional vibrations generated by the andullation technology in the horizontal position.

MATERIALS AND METHODS

Animals

In this study the experiments were performed on N.M.R.I. (Naval Medical Research Institute, Bethesda, Maryland, USA) female white mice, aged 6 to 8 weeks and weighing 28 to 30 grams. The investigations were approved by the local Animal Care Committee of the Vrije Universiteit Brussel. A lymphatic vascular mice model is used. The experiment concentrated on the popliteal and sacral lymph nodes in the posterior mouse paw and their connecting lymphatic vessel (cf. Fig. 1).

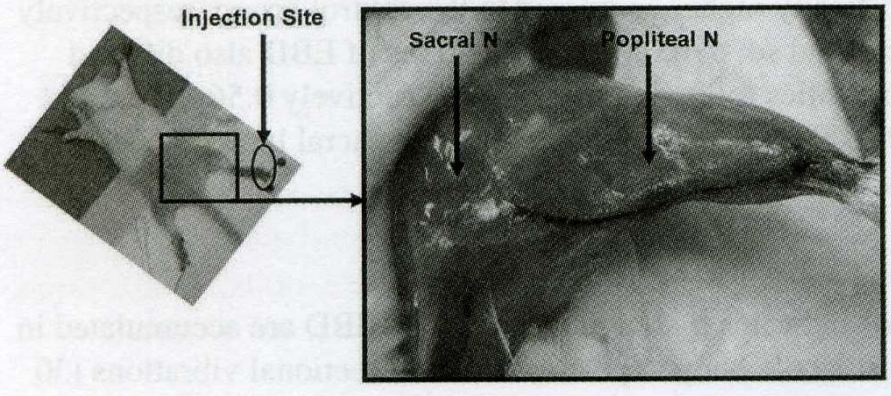
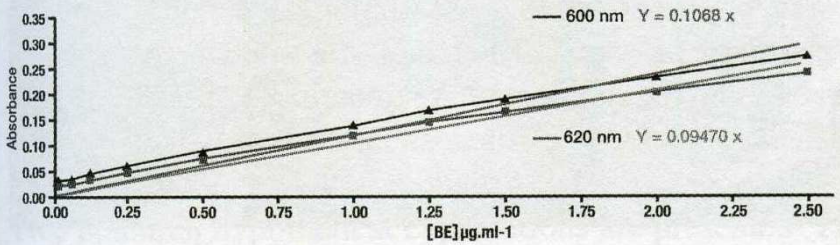


Fig. 1 - Popliteal lymph node, lymphatic vessel and sacral node (coloured with Patente Blue) in the mouse posterior paw.

Animal distribution was randomized in three groups of mice: 5 mice in the massage group, 10 animals in the vibration group and 10 mice in the control group. All animals were anaesthetized with urethane (2 mg/g i.p.). The hair on the posterior paws was shaved with electric clippers.

Assessment of accumulation of dye in the lymph nodes

In order to obtain quantitative values of lymphatic function (reabsorption and lymph flow), a spectrophotometric technique was chosen for analysis of the lymph node blueness. The technique is based on Beer Lambert's law describing a direct relationship between the absorbance of a monochromatic light crossing a homogenous dye solution and the amount of dye in this solution.
Harada and colleagues described a protocol to quantify Evans blue dye (EBD) extravasated into the skin (1971). Greco and his team applied this method for assessment of EBD in lymph nodes (2006). In both papers the quantitative analysis of EBD was performed at 620 nm. However, literature mentions other wavelengths for assessing the amount of dye. According to the absorbance spectrum of EBD, the best absorbance is obtained for a wavelength ranging from 600 to 630 nm. In a preliminary study, the authors evaluated the standard absorbance curves of EBD concentration ranging from 0 to 2.5 μg/ml at 600 and 620 nm to specify the best wavelength and the needed mathematical relationship (cf. graph. 1).



Graph 1 - Absorbance curves and linear regressions of EBD concentrations ranging from 0 to 2.5 ∝g/ml for 2 various wavelengths (600 and 620 nm). [Absorbance = a factor x EBD amount].

The results indicate no quality difference between these two wavelengths. They both present high but similar linear regressions in relation to small EBD concentrations. In this study 620 nm was selected to perform the EBD spectrophotometric determinations.

Experimental protocol

The study protocol was performed by the first author in each of the three groups of mice. Following anaesthesia and shaving, the animals were placed in an identical position on the same platform able to vibrate. Respecting five minutes of rest, an EBD solution (25 mg/ml, 20 ∝l) was injected subcutaneously in the dorsal side of the foot-pad over a period of three minutes. Bilateral injections were carried out in the massage group and the choice of the first injected foot-pad was randomized. Unilateral injections were performed in both the vibration and control groups where the injected foot-pad was randomized as well. Each group underwent a specific treatment during five minutes. The mice in the control group rested on the platform which was not induced to vibrate (cf. Fig. 2).
The animals in the vibration group underwent whole body vibrations at 30 Hz. The massage group received bilateral massage at the injection sites (cf. Fig. 3). At the end of the treatment animals were euthanized by cranial dislocation.

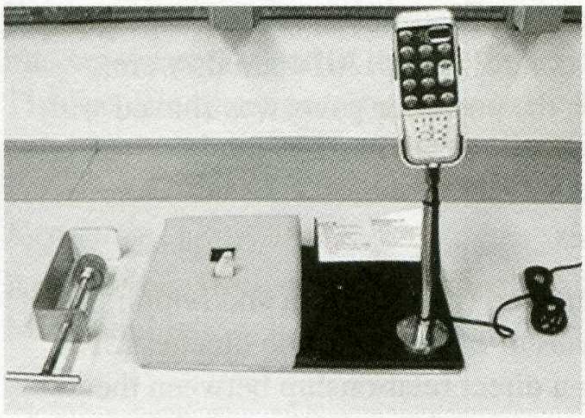


Fig. 2 - In the platform used during the experiments, a single motor of the andullation technology is built in to generate vibrations.

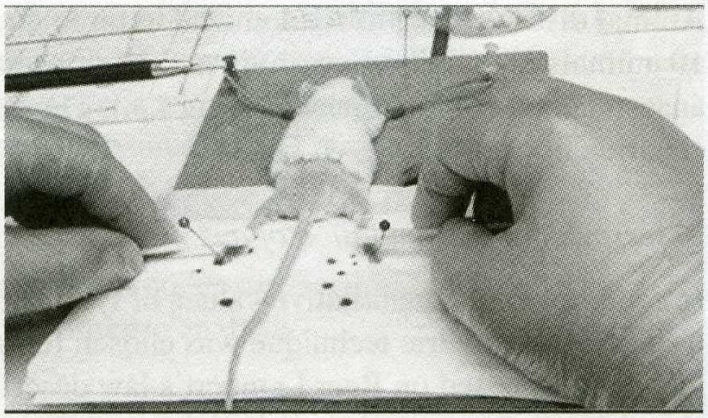


Fig. 3 - Bilateral massage at the injection site is performed with cotton buds by the same researcher respecting the same direction, speed and power.

The resections of the popliteal and sacral lymph nodes were realized in a randomized order (right or left first dissected posterior paw) for animals receiving local massage (cf. Fig. 4). EBD was extracted from the removed lymph nodes during 24 hours by immersing each of these nodes in a 7:3 mixture of acetone and 0.5% aqueous sodium sulphate solution (Harada, e. a., 1971). Spectrophotometric analysis of EBD was performed at 620 nm.

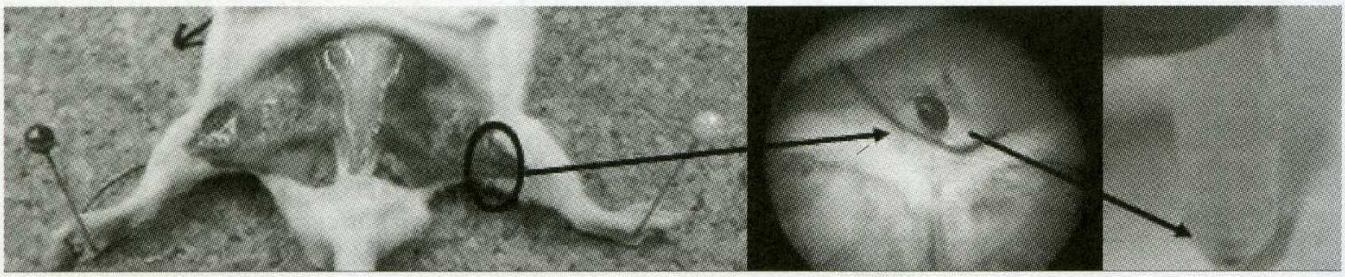


Fig. 4 - Popliteal lymph node resection realized after skin removal (left and middle images). Lymph node immersed in a specific solvent before spectrophotometric lymph node dye determination (right image).

RESULTS

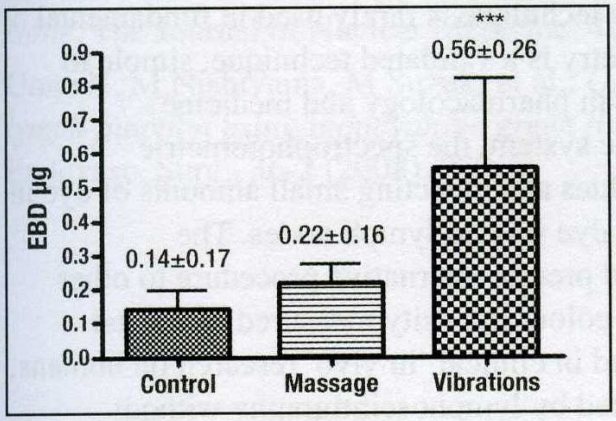
Accumulation of EBD in the popliteal lymph node.

In popliteal lymph nodes, the accumulated amount of EBD is significantly higher in the vibration group compared to the control group without intervention (respectively, 0.56 ± 0.26 and 0.14 ± 0.17, $p = 0.0005$). There is no difference when local massage is compared to no intervention (respectively, 0.22 ± 0.16 and 0.14 ± 0.17, $p = 0.3008$). A significant EBD accumulation in the popliteal nodes is observed when vibrations are applied rather than local massage (respectively, 0.56 ± 0.26, and 0.22 ± 0.16, $p = 0.0028$). Results are presented in graph 2.

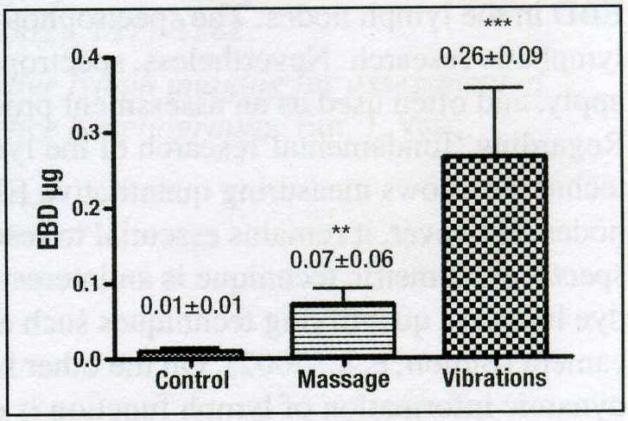
Accumulation of EBD in the sacral lymph node

Quantities ($\propto$l) of EBD in sacral lymph nodes following vibration are higher than in the control group (respectively, 0.26 ± 0.09 and 0.01 ± 0.01, $p<0.0001$) and are also

statistically different from the massage group (respectively, 0.26 ± 0.09 and 0.07 ± 0.06, $p<0.0001$).
Unlike the EBD quantities found in the popliteal lymph nodes and compared to control conditions, local massage increased the EBD amount in the sacral nodes (respectively, 0.07 ± 0.06 and 0.01 ± 0.01, $p = 0.0029$). Results are shown in graph 3.



Graph 2 - EBD amount (∝g) accumulated in popliteal lymph nodes
(mean and standard deviation).



Graph 3 - EBD amount (∝g) accumulated in sacral lymph nodes
(mean and standard deviation).

DISCUSSION

The presented experimental results indicate an important positive effect of multidirectional vibrations on the lymphatic system in the horizontal position. A short time exposure to whole body vibrations mechanically generated by the andullation technology improves lymph reabsorption and lymph flow.
Two potential hypothetical explanations are presented. The improved accumulation of dye in the lymph nodes could be explained from the mechanical effect of vibrations on mouse skin. In the present study, mice were placed in the ventral decubitus position to obtain the best contact between EBD injection site and the platform able to generate vibrations. The positional conditions allow the whole body to vibrate and the local skin to undergo massage at the injection site. Unno and colleagues injected Indocyanine Green Dye (ICG) and demonstrated that massage improves tracer evacuation from the injected area into the lymphatic system (2008). They also reported that massage increases the lymph flow in comparison to no manual intervention. In our experiment, EBD behaves similarly to ICG. When injected subcutaneously, EBD binds to endogenous proteins by a chemical sulfonation reaction (Sopelas, e. a., 2002). Bound EBD-proteins as well as free EBD are absorbed by the lymphatic vessels and transported along with the lymph flow. Sopelas found that in plasma or lymph EBD is bound to endogenous proteins by 66% (2002). This might explain why local massage on the injection site increases reabsorption and lymph flow of EBD and accelerates EBD accumulation in the lymph nodes as well. A similar mechanism explains the more impressive results quantified while vibrating the whole body.
A second hypothetical explanation relates to the kinetic behavior of EBD in lymph nodes. When EBD is injected, 20% of the total EBD contained in the lymph node is bound to proteins after short periods of time and found to be accumulated on cell surfaces of the reticulum endoplasmic system (RES) (Green, e. a., 2006). These data indicate the

possibility that massage or vibration could improve the accumulation of EBD-proteins on RES cell surfaces.
The authors favour the first explanatory hypothesis for their results. A specific lymphatic mouse model with 2 lymph nodes was opted for to obtain the best indirect appreciation of lymph flow. The large quantities of EBD present in the second lymph node reflect an increase of this flow. The higher the reabsorption and lymph flow, the higher the quantity of EBD in the lymph nodes. The spectrophotometric technique is rarely used in fundamental lymphatic research. Nevertheless, spectrophotometry is a validated technique, simple to apply, and often used as an assessment procedure in pharmacology and medicine. Regarding 'fundamental'research of the lymphatic system, the spectrophotometric technique allows measuring quantitative EBD-values and detecting small amounts of dye in nodes. However, it remains essential to resect the dye stained lymph nodes. The spectrophotometric technique is an interesting and precise alternative procedure to other dye blueness quantifying techniques such as blue colour intensity measured by digital camera (Sutton, e. a., 2002). On the other hand and in clinical 'in vivo' research on humans, dynamic information of lymph function is evaluated by lymphoscintigraphy without resection of part of the lymphatic circulatory system.

CONCLUSION

The aim of this study was to analyse the effects of short time exposures to multidirectional vibrations on the lymphatic system by spectrophotometry. Short time exposures to vibrations generated by andullation technology improve reabsorption and lymphatic flow. The results are encouraging and provide new data regarding the value of horizontal whole body vibrations for the lymphatic circulatory system. The findings arouse the necessity to consider vibrations as future potential biophysical options in the treatment of lymphatic diseases.

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