Research Article

Increase of drainage and immune functions of the lymph node as a result of phytostimulation in old age

Olga Gorchakova[1], Yurij Kolmogorov[1], Vladimir Gorchakov[1,2*], Natalia Kutafeva[2]

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Background: The most priority in medicine is the problem of providing an immune protection and increase in nonspecific resistance of an organism at elderly and senility age. First of all, search of ways of control of functions of lymphatic system is necessary for counteraction to aging. It is possible to make it by means of lymphotropic technologies of preventive medicine if to consider the concept of the lymphatic region.
Methods: In the experiment used 160 albino rats that were divided into groups of young and old animals. The mesenteric lymph node is chosen as a research object. We used the original herbal remedy (phytocomposition) to improve the function of the lymph node. We conducted a histologic research of mesenteric lymph nodes. We defined the content of trace elements (Mn, Fe, Cu, Zn, Se) in a mesenteric lymph node by means of the radiofluorescence analysis with use of the synchrotron radiation (RFA SI). Morphometric data processing was performed with licensed statistical software package StatPlus Pro 2009, AnalystSoft Inc.
Results: Age-related changes in mesocolic lymph node reflect the general process of ageing. These changes of lymph node are associated with reduction of structural and functional compartments and with excessive manganese content and deficiency of trace elements (iron, zinc, and selenium). There is a decrease in drainage and immune function of lymph nodes in the elderly and senile age. We have realized the idea to control the lymphatic system functions using phytotherapy. Phytotherapy provides improved drainage and immune functions of the lymph node by increasing the size of functional compartments, intensification of cellular proliferation and mitigating the deficiency of the main trace elements. There is the formation of new lymphoid follicles after phytostimulation.
Conclusion: Phytotherapy has a structural-modifying effect, which is important for improving the non-specific resistance of the body at the late stage of ontogenesis. This result is of practical importance for the optimization of endoecological rehabilitation.Key Words: Uterine fibroids, Menorrhagia, Pulmonary embolism, Deep venous thrombosis, Ovarian benign tumor.
Key words: Lymph node, Trace elements, Gerontology, Phytotherapy.

INTRODUCTION

The most priority in medicine is the problem of providing an immune protection and increase in nonspecific resistance of an organism at elderly and senility age. There is a disturbance of structure and function of lymphatic system with age. Involute processes in structures of lymphatic system reduce drainage of extracellular space and contribute to the development of immune insufficiency [1-3]. Emergence of the lymphatic theory of aging does relevant to consider changes in lymph nodes. The condition of the tissue microregion is connected with disturbance of drainage function of lymph nodes.

Scientists did not study completely a phenomenon of lymphatic system as instrument of providing a drainage and detoxication of endoecological space [4]. First of all, search of ways of control of functions of lymphatic system is necessary for counteraction to aging. It is possible to make it by means of lymphotropic technologies of preventive medicine if to consider the concept of the lymphatic region [1,5]. It is necessary to recognize as the most perspective direction of prevention of age changes lymphatic (lymphoid) systems. The idea of control of functions of lymphatic system is implemented in different preventive and therapeutic programs with use of non-drug methods of treatment. The greatest attention is deserved phytotherapy which is widely used in medicine. The lymphatic component of the mechanism of action of phytotherapy remains poorly studied [3,6,7]. There is an urgent need for scientific justification of expediency of phytotherapy for correction of age changes of lymphoid and lymphatic systems. The result has practical value for optimization of rehabilitation at a stage of late ontogenesis.

The purpose is studying influence of phytotherapy on structure and function of the lymph node which underwent age changes.

MATERIALS AND METHODS

The experiment is made on 160 white rats males of different age (3-5 months and 1.5-2 years) who conditionally divided into two groups of young and old animals [8]. Old animals are an adequate model of age-induced immune deficiency. The experiment on animals was carried out according to the international rules and norms (European Communities Council Directives of 24 November 1986, 86/609/EEC) with the general anesthesia of painful manipulations.

All the animals received a standard briquetted forage at free access to water. The mesenteric lymph node is chosen as a research object. We used biologically active herbal remedy (phytocomposition) which contained *Hedysarum theinum* Krasnob., *Bergenia crassifolia* (L.) Fritsch., *Rhodiola rosea* L., *Vaccinium myrtillus* L., *Vaccinium vitisidaea* L.), *Ribes nigrum* L., *Rosa majalis* Herrm., *Thymus serpyllum* L. and dietary fibers. The choice of medicinal plants is based on the principles of phytotherapy. Phytocomposition is a source of bioflavonoids, microelements and other biologically active agents which have adaptogenic and lymphotropic effects [3,6,7]. Action mechanism of bioflavonoids and microelements are connected with activation and proliferation of immunocompetent cells [9-12]. The daily dose of phytocomposition was 0.1-0.2 g/kg, and it was added to a standard forage by an animal of different age within one month.

We conducted a histologic research of mesenteric lymph nodes. Lymph nodes fixed in 10% neutral formalin. We adhered to the classical scheme of dehydration and embed in paraffin with preparation of histologic sections. Histological sections of lymph nodes painted hematoxylin and eosine, azury and eosine, Masson's trichromatic stain. The morphometric analysis of structural components of a lymph node was carried out by means of a morphometric grid [13] which imposed on a slice of lymph node. We counted the number of crossings of a grid which fall on all cut

[1]*Institute of clinical and experimental lymphology, 2, Timakov str., Novosibirsk, 630060, Russia*

[2]*Novosibirsk state university, 2, Pirogov str., Novosibirsk, 630090, Russia*

[*]*Correspondence: Vladimir Gorchakov, MD, Institute of clinical and experimental lymphology, P.O. Box 47, Novosibirsk-60, 630060, Russia, E-mail: vgorchak@yandex.ru*

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and separately on each of structural components of a lymph node (capsule, cortical plateau, lymphoid follicles, paracortex, medullary cords, lymphatic sinus) with recalculation in percent.

We defined the content of trace elements (Mn, Fe, Cu, Zn, Se) in a mesenteric lymph node by means of the radiofluorescence analysis with use of the synchrotron radiation (RFA SI) at the VEPP-3 station of Institute of nuclear physics of G.I. Budker (Novosibirsk). Energy of a monochromatic bunch was 17 keV. Quantitative appraisal of data of an emission spectrum of lymph nodes was executed with «the external standard».

Statistical data processing was performed with licensed statistical software package StatPlus Pro 2009, AnalystSoft Inc. Data were expressed as average arithmetic with definition of a standard (mean square) error. Belonging to normal distribution was defined when calculating criterion of Kolmogorov-Smirnov and the accompanying indicators. In work the correlation analysis with definition of a correlation coefficient of Brave-Pearson is used. A P value <0.05 was considered statistically significant.

RESULTS

The structure of a lymph node changes with age. Comparative analysis showed age differences in a structure of a lymph node of old and young animals. There is an increase in the areas of the capsule (in 1.6 times), medullary cords (in 2.1 times) and reduction of subcapsular and medullary sinuses (in 1.3 and 1,8 times respectively), the cortical plateau (in 2.1 times), lymphoid follicles with the germinative center (in 1.4 times), by 12% of a paracortex for with age (Table 1).

Table 1: The area of structural and functional zones of a mesenteric lymph node of young and old animals and after intace of bioactive phytocomposition (BAP), %.

Structures of lymph node	Young animals	Old animals without intake of BAP	Old animals with intake of BAP
	1	2	3
Capsule	5.72 ± 0.19	9.43 ± 0.45*	9.04 ± 0.48
Subcapsular sinus	4.57 ± 0.17	3.38 ± 0.27*	4.50 ± 0.22°
Cortical plateau	7.74 ± 0.31	3.78 ± 0.24*	4.28 ± 0.24°
Lymphoid follicle without the germinative center	4.18 ± 0.17	3.32 ± 0.22*	4.32 ± 0.21°
Lymphoid follicle with the germinative center	5.69 ± 0.19	3.03 ± 0.26*	4.62 ± 0.46°
Paracortex	16.02 ± 0.56	14.29 ± 0.54	12.28 ± 1.11
Medullary cords	10.55 ± 0,24	22.03 ± 0.72*	16.63 ± 1.62°
Medullary sinus	7.31 ± 0.29	3.89 ± 0.35*	5.61 ± 0.61°

Note: level of the statistical importance of distinctions–*P_{1-2}<0.05; °P_{2-3}<0.05.

n=20: number of rats in each group

Table 2: The content of trace elements in a mesenteric lymph node of young and old animals and after intake of bioactive phytocomposition (BAP), mkg/g.

Trace elements	Young animals	Old animals without intake of BAP	Old animals with intake of BAP
	1	2	3
Mn	2.15 ± 0.13	2.71 ± 0.14*	2.97 ± 0.20
Fe	254.8 ± 20.66	182.6 ± 14.33*	241.2 ± 22.57°
Cu	6.48 ± 0.47	5.29 ± 0.35	7.22 ± 0.22°
Zn	68.71 ± 2.52	57.27 ± 1.72*	65.87 ± 2.09°
Se	1.38 ± 0.05	1.14 ± 0.06*	1.24 ± 0.07

Note: level of the statistical importance of distinctions–*P_{1-2}<0.05; °P_{2-3}<0.05.

n=20: number of rats in each group

Cortical substance of a lymph node undergoes the most expressed changes because of age involution. The most part of structure of a lymph node is provided by a reticular stroma. Narrowing of lymphatic sinuses indicates decrease in drainage activity of a lymph node. Reduction of the area of structural and functional zones, especially lymphoid follicles with the germinative center, indicates decrease in proliferative processes and immune potential in a lymph node at elderly and senile age. The direct dependence between saturation immunocompetent cells of structurally functional zones of a lymph node and type of an immune response takes place. The immune response is lowered as on humoral, and cellular type at old animals.

Earlier pathophysiological mechanisms of age involution of a lymphoid tissue were not considered in interrelation with a trace element profile of a lymph node. As showed researches, change of structurally functional zones of a lymph node depends on the content of trace elements [14]. The lymph node has an age-induced imbalance of trace elements. We defined excess of content of manganese (in 1.3 times) and the deficit of content of iron (in 1.4 times), zinc (in 1.2 times) and selenium (in 1.2 times) in a lymph node of old animals (Table 2).

Content of copper in a lymph node of old animals tend to reduction, but does not give reliable distinctions with young animals. The imbalance of microelements affects regulation of processes of proliferation and a differentiation of lymphoid cells. The activity loss of cellular proliferation is connected with decrease of concentration of trace elements which are components of enzymes [14-16].

Phytocorrection causes positive changes of structural and functional zones of a lymph node of old animals. There is an increase in the sizes of a subcapsular sinus (in 1.3 times) and a medullar sinus (in 1.4 times), areas of lymphoid follicles with germinative center (in 1.6 times). Besides, we noted decrease in 1.3 times of the area of medullary cords and tendencies to decrease by 16% of a paracortex (Table 1). Change of intranodal zones is followed by compaction of a lymph node after phytotherapy at old animals. There is an increase in size of a cortical and medullar ratio. Phytotherapy increases intensity of an immune response on humoral type as the area of B-dependent compartment of a lymph node changes (Table 1).

The number of lymphocytes increases in 1.2-1.3 times in all structural and functional zones of a lymph node after phytotherapy. The number of

plasmocytes increases in medullary cords of lymph node at preservation of number of blasts after phytotherapy. Strengthening of a limfoproliferation after phytotherapy leads to formation of temporary lymphoid structures–lymphoid follicles. The similar ectopic focus of accumulation of lymphoid cells (lymphoid follicles) are called «tertiary lymphoid organs». The ectopia of lymphoid follicles is noted in a subcapsular zone and medullary substance of lymph nodes (Figures 1 and 2) and outside lymph nodes (Figure 3) after phytotherapy at old animals. It is experimentally proved that phytotherapy is capable to induce formation of new lymphoid structures (neolymphogenesis) with strengthening of immune function of a lymph node.

We established a phytotherapy contribution to provision with lymph node trace elements at a late stage of ontogenesis. Phytotherapy leads to increase iron (in 1.3 times), copper (in 1.4 times), zinc (in 1.2 times) in a lymph node of old animals that is sufficiently near to the content of trace elements at young animals. Content of manganese remains excessive in a lymph node at a late stage of ontogenesis in comparison with young animals. Content of selenium fluctuates at the lower bound of an indicator. The created trace element profile after phytotherapy positively affects cellular proliferation and dimension of compartment of a lymph node.

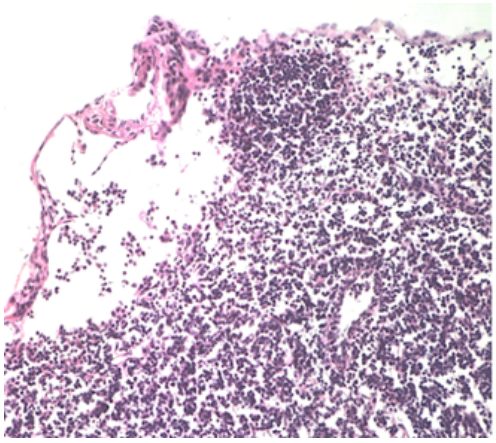

Figure 1: Ectopia of lymphoid follicles in a subcapsular zone a mesenteric lymph node. Old animals. Phytotherapy. Hematoxylin and eosin stain (Enlarged image ×240).

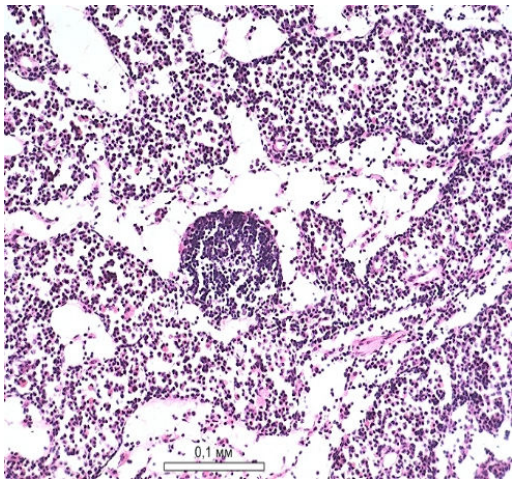

Figure 2: Ectopia of lymphoid follicles in a medullary substance a mesenteric lymph node. Old animals. Phytotherapy. Hematoxylin and eosin stain (Enlarged image ×240).

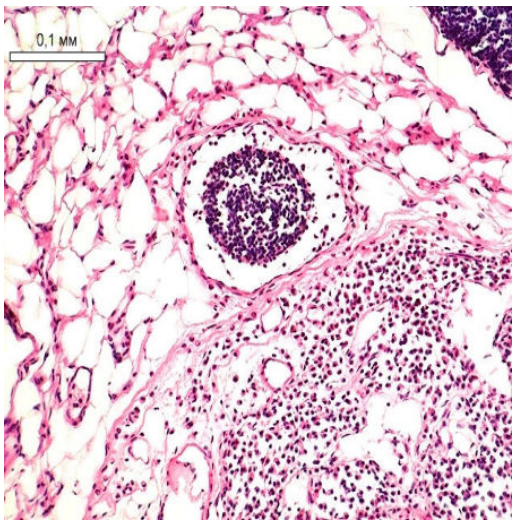

Figure 3: Ectopia of lymphoid follicles outside a mesenteric lymph node. Old animals. Phytotherapy. Hematoxylin and eosin stain (Enlarged image ×240).

The characteristic of changes of structure, cells, trace elements of lymph node after phytotherapy indicates increase in drainage and immune functions that it is the important fact for elderly and senile age.

Phytotherapy is an element of «background» therapy in sanatorium prevention and rehabilitation of pathology taking into account age. Herbal remedy have a number of the indisputable advantages caused by lack of side effects, biological affinity to a human body, influence on the interfaced systems and organs including on lymphatic system, long and steady effect. Inclusion of phytotherapy promotes increase in efficiency of the carried-out treatment and rehabilitation that is proved by experience in sanatorium practice [1,3,4].

DISCUSSION

The fact of decrease in drainage and immune functions of a lymph node has a causal relationship with change of its structure and trace element balance when aging. It is the destabilizing moment of adaptation and compensatory processes at advanced age and increases risk of development of a polymorbid state. Undeniable condition is normal functioning of a lymph node for ensuring the immune status and to counteraction to aging [1,17,18]. Use of original phytocomposition allows to reach structural and functional unity of a lymph node.

Original phytocollecting solves problems of functional orientation. First of all, it is connected with the structural and modifying effect of phytodrug when aging of a lymph node takes place. The size of compartment of a lymph node depends on the numerical density of lymphoid cells and determines an immune response by cellular or humoral type. T lymphocytes are concentrated in a paracortex (a thymic-dependent zone), B-lymphocytes are localized in lymphoid follicles and medullary cords (a thymic-independent zone). The cytocomposition with different dimension of compartment reflects stages of transformation of a lymphoid parenchyma of a lymph node both when aging, and after influence of herbal remedy [19].

The phytotherapeutic effect consists in completion of deficit of microelements, bioflavonoids and other biologically active agents, optimization of structure and function of a lymph node according to the principles a lymphosanitation, a lymphoprotection and a lymphostimulation [1,3,6,17].

Herbal remedy is a source of trace elements. Increase in concentration of microelements at intake of vegetable drug is favorable for normal functioning of lymphoid cells [7,15,16]. Trace elements have selective effect on functions of immune system that is shown in change of

proliferation of immunocompetent cells [10]. Often change of trace elements contents is the reason of cellular changes in lymphoid follicles. So, copper influences processes of formation of immunocompetent cells [10,20] and, therefore, on the size of functional compartment.

Phytotherapy correction structure of the lymph node which underwent senile changes. Phytotherapy provides a progressive morphogenesis due to strengthening of proliferation and a differentiation of lymphoid cells, increase in structural and functional zones and sinus system at a late stage of ontogenesis. We assume that the microelement profile and proliferation of lymphoid cells gives positive lymphotropic effect of phytotherapy on structure and function of a lymph node of old animals.

Emergence of lymphoid follicles in tissue or organs is morphological expression of degree of immune tension [21]. One of effects of the strengthened cellular proliferation is formation of new lymphoid structures (lymphoid follicles) inside and outside of a lymph node. The lack of CD38+ of cells is the certificate in favor of lymphoid follicles as only plasmocytes and plasmoblasts of medullary cords come to light when using anti-CD38 antibodies [22]. Neolymphoid aggregates are necessary for implementation of an adaptive immune response [23-27] that is important for neogenesis of lymph nodes [25,28,29]. It is a refute arguments on loss of regenerative ability of a lymphoid tissue when aging. Formation of lymphoid follicles is result of phytostimulation of an immune response [22]. It is a fact in evidence increase drainage and immune functions of a lymph node as a result of phytotherapy of old animals [3,6,17,30,31].

We believe that phytotherapy provides an active circulation of the lymph that provides structural integrity of the functional compartment of a lymph node necessary for performance of drainage and immune functions, despite the remaining sclerotic and atrophic processes when aging. We consider that use of original phytoremedy at elderly and senile age is reasonable. Phytotherapy is means implementation of technology of recovery correction of the lymph node which underwent age changes that provides increase in nonspecific resistance of an organism.

CONCLUSION

The age-caused changes of a mesenteric lymph node accompany the general process of aging, and they are connected with reduction of the sizes of structural and functional zones against the background of redundancy of manganese and deficiency of iron, zinc and selenium that reduces functional activity of a lymphoid tissue at elderly and senile age. Original herbal drug has the lymphotropic and structural modifying effect. Phytotherapy provides increase drainage and immune functions due to recovery of structure with increase in the size of compartments, strengthening of a lymphoid proliferation and elimination of deficit of trace elements in a lymph node at a late stage of ontogenesis. It leads to increase in nonspecific resistance of an organism at elderly and senile age. The lymphotropic phytotechnology is positioned as a possibility of improvement of quality of life and maintaining health through optimization of structure and functions of a lymph node in relation to elderly and senile age.

AUTHOR'S CONTRIBUTIONS

Conseption and design: Olga Gorchakova, Vladimir Gorchakov. Analysis and interpretation: Olga Gorchakova, Vladimir Gorchakov, Yurij Kolmogorov. Data collection: Yurij Kolmogorov, Olga Gorchakova, Natalia Kutafeva. Writing the article: All authors. Critical revision: All authors. Final approval of the article: All authors. Overall responsibility: Vladimir Gorchakov.

CONFLICTS OF INTEREST

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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