ISSN: 0974-8369

Biology and Medicine

International, Open Access

Available online at: www.biolmedonline.com

Biology and Medicine

Isakina *et al.*, Biol Med (Aligarh) 2015, 7:2
http://www.biolmedonline.com

Research Article Open Access

Influence of Potassium Hyaluronate on the Content of Lysophospholipids and Free Fatty Acids in Damaged Somatic Nerves of Rat

Marina Vladimirovna Isakina*, Nadezhda Victorovna Revina, Victor Vasilevich Revin

Ogarev Mordovia State University, 26b, Ulianova Street, Saransk 430032, Republic of Mordovia

Abstract

Changes in lipid composition and the state of the membranes of somatic nerves of rats were studied in case of damage under the action of potassium hyaluronate. The study has shown that the introduction of the drug contributes to a less manifested accumulation of lysophospholipids and free fatty acids, and to decreased activity of A_2 phospholipase. Using the method of Raman scattering spectroscopy, it was found that potassium hyaluronate increases the orderliness of fatty acids that is an indicator of membrane viscosity.

Keywords

Lysophospholipids; Free fatty acids; Potassium hyaluronate; Damage to somatic nerves; Raman scattering spectroscopy

Introduction

Currently, a lot of attention is paid to the problem of recovering functions of damaged peripheral nerves. Despite numerous studies of neurodegenerative processes occurring in somatic nerves in case of damage, the mechanisms of their development remain poorly understood [1]. In recent decades, various biologically active substances, which promote the regeneration of damaged nerve tissue, have been actively searched for. One of the most promising substances used in reconstructive surgery and tissue engineering is hyaluronic acid [2-4]. It is reported that the high molecular weight form of hyaluronic acid ensured proliferation and self-renewal of neuronal stem cells [5]. It is possible that by accelerating regenerative processes, the hyaluronic acid helps to restore properties of cell membranes, with lipids being one of the main components [6].

Numerous experimental data about the role of lipids in passing agitation through nerve fiber [7-9] have been accumulated. It has been established that nerve degeneration caused by cutting off is accompanied by changes in the lipid composition of the nerve fibers [8,9]. In recent years, more and more data have appeared about regulatory effects of lysophospholipids (LPL) which work as mediators that cause a variety of cellular responses. A special place is occupied by lysophosphatidic acid, lysophosphatidylcholine (LPC), and a number of lysosphingolipids [10-12]. They are involved in regulating activity of the majority of enzymes associated with membranes, development of physiological reactions and pathological processes [10,13,14]. In this regard, the aim of our work was the study of the influence of hyaluronic acid on the changes in lipid composition and the state of cell membranes in degenerative processes in rats' somatic nerves.

Methods

The study was focused on the sciatic nerves of white outbred rats weighing 200-250 g, and on the lipids extracted from them. To create a model of pathology, the sciatic nerve was cut at the level of rat's midthigh. In animals of the same group anesthetized with diethyl ether, the sciatic nerve was exposed, suture was applied to it, and the nerve was cut. In animals of the second group, solution of potassium hyaluronate (PH) (hyaluronic acid potassium salt from human umbilical cord, Sigma) was applied to the proximal and distal ends of the severed nerve at concentrations of 2 mg/kg, 17 mg/kg, and 30 mg/kg. At the end of preparation, the wound was sewed up. The proximal and distal ends of the nerve were removed after 12 h, 1 day, 3 days, 7 days, and 30 days, and were placed in Ringer's solution. Intact animals were used for reference. Lipids were removed from the nervous tissue according to the method of Blaye-Dyer [15]. Phospholipids were separated using the method of one-dimensional chromatography on silica gel in the chloroform/methanol/ammonia/water (65:30:4:2) [16] system of solvents. Phospholipids were separated and quantitatively determined using densitometric method in the CAMAG TLC Scanner 4 automated complex (Switzerland). For separating free fatty acids (FFA), the heptane/diethyl ether/glacial acetic acid (60:40:1 by volume) system was used [17]. Fatty acids (FA) were methylated with a solution of boron trifluoride in methanol [18]. The activity of phospholipase A_2 (PL A_2) was determined by accumulation of FFA, composition of which was analyzed using a Shimadzu GS 2010 (Japan) gas chromatograph [9]. Separate fractions of phospholipids were identified using Rf values, specific coloring agents and markers (Supelco). Change in the status of the membranes was detected by a Renishaw Raman spectrometer (UK). Statistical processing was performed using Student's *t*-criterion.

Results and Discussion

It is known that in case of nerve damage, its central and peripheral segments undergo various changes [19]. It is therefore of interest to study the depth of development of degenerative processes and the influence of PH on the regeneration processes in each of the segments of damaged nerve fibers. It was shown that LPL formed during activation of phospholipase A_2 are involved in the development of

***Corresponding author:** Isakina MV, Ogarev Mordovia State University, 26b, Ulianova Street, Saransk 430032, Republic of Mordovia

Received: January 22, 2015; **Accepted:** February 15, 2015; **Published:** April 13, 2015

Citation: Isakina MV, Revina NV, Revin VV (2015) Influence of Potassium Hyaluronate on the Content of Lysophospholipids and Free Fatty Acids in Damaged Somatic Nerves of Rat. Biol Med (Aligarh) 7(2): BM-071-15, 4 pages.

many pathological processes [20]. On this basis, we first investigated the change in the content of LPL in the proximal end of the sciatic nerve after cutting. The research showed that in the intact sciatic nerves of rats, the average LPL content was 0.03 μg P_{LPL}/μg P_{PL}. However, in as little as 12 h after the injury, an increase is observed in the content of LPC and lysophosphatidylethanolamine (LPEA) in the proximal end of the nerve, which is 1.3 times higher as compared to the reference. The increase in the duration of the damaging interference is accompanied by increasing level of phospholipids lysophorms with the maximum accumulation on the third day of the experiment. With that, 7 days after the injury, a tendency is observed to decreasing LPC and LPEA content, nevertheless their level exceeds the one in the reference group by 33.3% and 12.5%, respectively. With increasing the post-surgical period up to 30 days, a further decrease in the level of LPL is observed, but their content within this period is still 1.9 times higher than the reference values in the average (Figures 1 and 2).

Apparently, accumulation of LPL after damaging the nerve is due to the increased activity of PL A_2, which catalyzes hydrolysis of phospholipids mainly in sn-2 position that is characteristic of polyunsaturated FA [9]. To confirm our assumptions, we performed a series of experiments for determining the phospholipase activity in the damaged nerve conductor. In a series of experiments with the damage, the maximum enzyme activity was found on the seventh day of the experiment and reached 221 μg of FA/mg of protein*hour. Thirty days after the injury, the activity of PL A_2 decreased 12 times, but still exceeded the reference value by 110%. Activation of FL A_2 can be judged by the content change of FFA, which is one of the lipid metabolites resulting from hydrolysis of PL A_2. According to the results of the research, 12 h after the trauma, an increase in the proportion of FFA was observed due to a 2.1 times increase in the content of long-chain fatty acids, as compared to the reference group. However, the maximum accumulation of FFA occurred on the third day of the experiment. Increasing the duration of damaging effect to 30 days was accompanied by a decrease in FFA level. However, FFA content still considerably differs from the reference values, exceeding its 2 times on average (Figure 3).

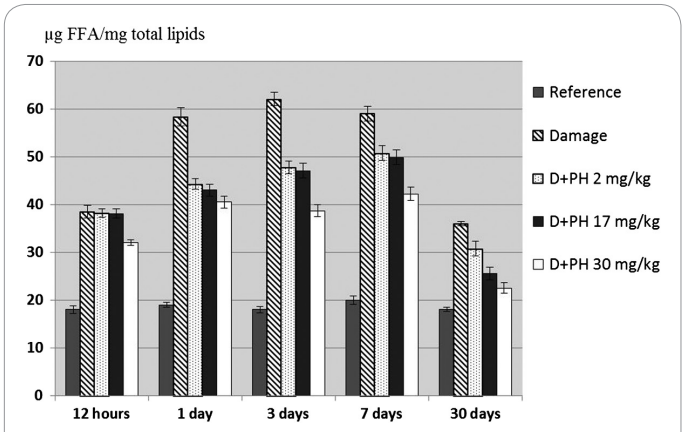

Figure 3: The effect of PH on FFA content in the proximal end of rat's sciatic nerve.

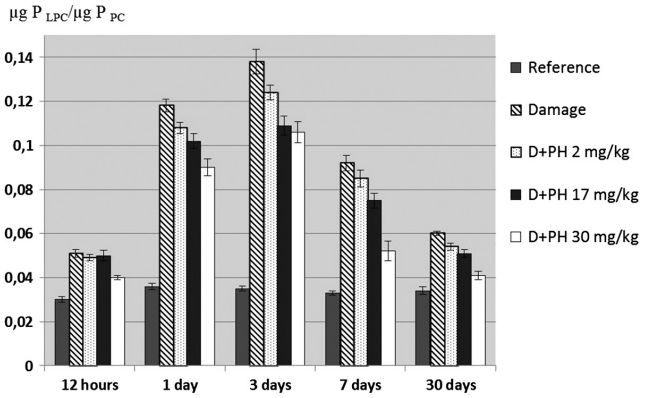

Figure 1: The effect of PH on changing the content of LPC in the proximal end of a nerve after cutting: D+PH 2 mg/kg – damage + PH in concentration 2 mg/kg, μg P_{LPC}/μg P_{PC} – μg of inorganic phosphorus LPC/μg of inorganic phosphorus PC (phosphatidylcholine).

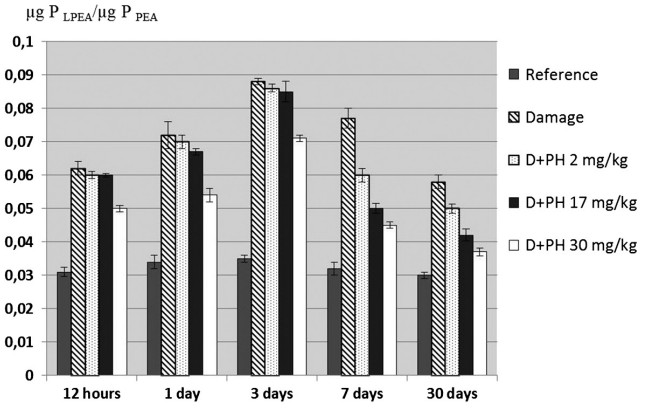

Figure 2: The effect of PH on changing the content of LPEA in the proximal end of the nerve after cutting.

Thus, the increase in FFA content by the third day of the experiment followed by its reduction by the 30th day correlated with the similar change in the amount of LPC, LPEA in the nerve after cutting. This indicates the direct relationship between the changes in the content of LPL and FFA.

As already mentioned, hyaluronic acid is an efficient biologically active compound that affects regeneration processes [5] and nerve conduction [21]. It has been established that PH in low concentrations has virtually no effect on changing the content of LPL in the proximal part of the nerve conductor. Veracious reduction in their level is observed in case of using the drug at a concentration of 30 mg/kg. Moreover, the most pronounced effect of PH is observed long time after the nerve damage. So, on the first, third, and seventh day of the experiment the content of LPC, LPEA in a series of experiments with PH is reduced as compared to cutting by 24.4 on average: 21.3% and 42.6%, respectively. It should be noted that with increasing postoperative period up to 30 days, the content of LPL in the variant with PH is reduced 1.6 times on average, as compared to the damage, and does not significantly differ from the reference (Figures 1 and 2). In a series of experiments with introducing PH, the phospholipase activity also increased, but to a lesser degree, as compared to the injured

nerve without the influence of the drug. The minimum level of enzyme activity is observed in animals that received injections of PH at the rate of 30 mg/kg, the phospholipase activity is reduced 2.4 times. The level of FFA in a series of experiments with the use of the preparation also changes substantially. The most pronounced effect of the preparation is manifested in case of its maximum concentration after longer periods after cutting. So, by the 30th day of the observation, the amount of FFA is reduced by 37.5%, as compared to the damage, and is slightly higher than the reference value (Figure 3).

From the obtained data, it can be assumed that LPC accumulation after cutting the nerve is associated with the increased activity of phospholipase A_2. This is evidenced by data about PL A_2 participation in the process of early degradation of myelin in course of Wallerian degeneration of nerves in rats and mice [22,23] and in sciatic nerves of frogs [24]. Detergent action in relation to the membranes is possessed not only by LPL but also by FFA, which are potent effectors of physiological and biochemical processes [25]. Therefore, an increase in FFA concentration during the initial period after cutting of the nerve indicates development of degenerative processes in the proximal end of the nerve conductor. However, with increasing the post-surgery period, a tendency is observed to decreasing LPL and FFA. This is consistent with the literature data, indicating that the degeneration ends on fifth and seventh day after the nerve injury [8], and reparative regeneration of the myelin sheaths of nerve fibers actively runs until the 30th day after nerve compression and lasts up to 50 days of the experiment [19]. It is known that hyaluronic acid reduces hydrolysis of phospholipids, reducing activity of secretory PL A_2 in case of a sharp tissue damage [26]. In addition, it plays an important role for protecting phospholipids in the synovial fluid from lysis by PL A_2 [27]. Considering the literature data and results of our own research, one can make the assumption that the manifestation of lipoic properties of PH is implemented through regulation of the activity of the membrane-bound phospholipase A_2.

It is known that in the distal part of the nerve conductor, due to disappearance of central regulation, an amplification of degenerative processes occurs, including oxidation in the absence of regulatory mechanisms [9]. On this basis, we performed comparative analysis of changes of the lipid composition in the proximal and distal ends of the nerve after the damage and introduction of PH. The study showed that more pronounced degenerative processes throughout its duration occur in the distal end of the nerve. With that, the minimum level of LPL, same as in the proximal end, was observed in animals that received injections of PH in its maximum concentration. However, on the third and seventh day of the observation, PH had a stronger positive effect in the distal end of the nerve, as compared to its proximal segment, and caused average reduction in LPL amount 2 times as compared to the damage. By the 30th day of the experiment, the content of LPL virtually did not differ from the reference values. In the fraction of FFA, the similar dynamics was observed: after 3 days, the amount of FFA reduced 2.4 times, and with increasing duration of the experiment up to 30 days, the level of FFA decreased 3.6 times in a series of experiments with the use of the preparation in the maximum concentration. Comparing the extent of changes in the studied sections of the nerve, one should note that in the distal end of the nerve they are more pronounced, and it is in this variant of the experiment that PH has its stabilizing effect on recovery of LPC and FFA level to the greatest extent. Probably, connection the central nervous system remains in the proximal section, and due to the existence of compensatory mechanisms, degenerative processes flow less intensively in the lipid phase of somatic nerves.

It is known that the composition and the state of lipids largely influence the functional state of excitable formations [28]. Therefore, in the next phase of the study using the Raman scattering spectroscopy method, we evaluated the orderliness of fatty acids, which indicates viscosity of the membrane [9]. It was found that the ratio of intensities of peaks I_{1445}/I_{1300} reflects the orderliness of fatty acid chains that form the hydrophobic layer of the membrane [29,30]. With reduction of this indicator, the orderliness decreases, which is accompanied by a decrease in the membrane viscosity. During the experiment, it was shown that the ratio of intensities of peaks I_{1445}/I_{1300} reduces till the seventh day of observation by 77% as compared to the reference group. However, increasing the time of the damaging effect up to 30 days leads to an increase in the orderliness of FFA residues in the membrane 1.7 times, which indicates an increase in the membrane micro-viscosity in the sciatic nerve. In a series of experiments by introducing PH in low concentrations, the I_{1445}/I_{1300} ratio virtually does not change. Its veracious increase is observed in case of using the preparation at a concentration of 30 mg/kg. And on the third and seventh day of observation, the value of the index increases, as compared to the day of the damage, by 50% and 46%, respectively. By the 30th day of the experiment, the ratio of peaks I_{1445}/I_{1300} virtually does not differ from the reference. The orderliness of fatty-acid chains in the hydrophobic layer of the membrane can also be judged by the indicator of intensities of the I_{1650}/I_{1445} bands, which characterizes the saturated to unsaturated fatty acid ratio. The increase in the ratio of I_{1650}/I_{1445} peaks reflects the increased content of unsaturated fatty acids [29,30]. The experiment showed that the degree of fatty acids desaturation increased till the seventh day of the experiment by 241% as compared to the reference, and after 30 days, the value of this indicator reduces, but still exceeds the reference value 1.7 times on average. In a series of experiments with PH, this figure is reduced already on the first day of observation, and with increasing the experiment duration up to 30 days, its value is close to the reference. Thus, the viscosity of myelinic nerve fiber correlates with the change in the degree of fatty acids desaturation. Accumulation of LPL in case of nerve damage is straining orientation of the fatty acid chains of phospholipids, which is accompanied by loosening and disruption of the membranes structure, which in its turn contributes to a reduction in the micro-viscosity of the myelinic nerve fiber [9].

Conclusion

As we can see from the results obtained, the introduction of PH enhances regenerative processes in the injured nerve: activity of phospholipase A_2 decreases, orderliness of the membrane increases, and the ratio of saturated/desaturated fatty acids changes toward desaturated fatty acids. Considering the results of our own research, and the data from literature, it can be assumed that hyaluronic acid accelerates regeneration systems in nerves. It is quite possible that one of the mechanisms of this process is implemented via phospholipase A_2, activation of which is the key factor in degenerative and regenerative processes [22,24,27].

References

1. Minasyan AL, Aznauryan AV, Meliksetyan IB, Chavushyan VA, Sarkisyan DS, *et al.* (2011) Development of neurodegenerative processes in flexor and extensor branches of the sciatic nerve after crushing; regeneration under the influence of peptide enriched with proline. Neurochemistry 28(4): 315-322.

2. Sevast'yanov VI (2009) Biomaterials, medications delivery systems and bioengineering. Bulletin of Transplantology and Artificial Organs 11(3): 69-78.

3. Rakhmatullin R, Burlutskaya O, Adelshina L, Burtseva T (2011) "Geomatrix" nanostructured material. Doctor 5: 22-24.

4. Rakhmatullin R, Burlutskaya O, Gilmutdinova I, Adelshina L, Burtseva T (2011) Studying biological compatibility of the new "Geomatrix" biomaterial. Doctor 6: 32-34.

5. Back SA, Tuohy TM, Chen H, Wallingford N, Craig A, *et al.* (2005) Hyaluronan accumulates in demyelinated lesions and inhibits oligodendrocyte progenitor maturation. Nature Medicine 11: 966-972.

6. Torigoe K, Tanaka HF, Ohkochi H, Miyasaka M, Yamanokuchi H, *et al.* (2011) Hyaluronan tetrasaccharide promotes regeneration of peripheral nerve: *In vivo* analysis by film model method. Brain Research, 1385: 87-92.

7. Revin VV, Yudanov MA, Revin ES, Maksimov GV, Grunyuskin IP (2006) Study of changes in the content of diacylglycerol in nerve excitation. Biochemistry 71(10): 1354-1359.

8. Revin VV, Yudanov MA, Maximov GV (2006) The composition of lipids in somatic nerves of rats under the action of damaging factors. Bulletin of Experimental Biology and Medicine 142(8): 155-157.

9. Revin VV, Revina ES, Devyatkin AA, Gromova NV (2012) The Role of Lipids in Functioning of Excitable Biological Membranes. Saransk: Publishing House of the Mordovia University, p. 220.

10. Torkhovskaya, TI, Ipatova OM, Zakharova TS, Kochetova MM, Khalilov EM (2007) Cell receptors for lysophospholipids as promoters of signal effects (review). Biochemistry 72(2): 149-158.

11. Dyatlovitskaya EV (2007) The role of lysosphingolipids in regulation of biological processes. Biochemistry 72(5): 596-602.

12. Berdichevets IN, Tjazhelova TV, Shimshilashvili HR, Rogaev EI (2010) Lysophosphatidic acid, a lipid mediator with numerous biological functions: Biosynthesis ways and mechanism of action. Biochemistry 75(9): 1213-1223.

13. Gribanov GA (1991) Features of the structure and the biological role of lysophospholipids. Problems of Medical Chemistry 37(4): 2-10.

14. Prokazova NV, Zvezdina ND, Korotayeva AA (1998) The influence of lysophosphatidylcholine on transmitting transmembrane signal inside a cell. Biochemistry 63(1): 38-46.

15. Bligh E, Dyer W (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911-917.

16. Scharer C (2001) Diplomarbeit Vergleich von HPLC-ELSD und moderener TLC in der heutigen Phospholipid-Qualiatskontrolle. Fachhochschule beider Basel, p. 48.

17. Biological membranes. In Methods, Findlay J, Evance U (Eds.), 1990. Moscow: Mir, p. 424.

18. Morrison WR, Smith LM (1964) Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipid Research 5: 600-608.

19. Arkhipov SS, Ragimov IS, Mechitov AR, Chelyshev YA (2009) Satellites cells of sensitive neurons in different types of injuries of the sciatic nerve of rats. Morphology 135(3): 29-34.

20. Yefremova AS, Zinchenko VP (2008) Involvement of calcium-independent phospholipase A_2 in regulation of Ca^{2+} signal induced by calmodulin inhibitor of in thymocytes of rats. Biological Membranes 25(4): 292-300.

21. Samoilenko AV (2004) Hyaluronic acid in treatment and prevention of ciliochoroidal detachment. Glaucoma: Research and Clinical Journal 4: 22-26.

22. Paul JA, Gregson NA (1992) An immunohistochemical study of phospholipase A_2 in peripheral nerve during Wallerian degeneration. Journal of Neuroimmunology 39: 31-48.

23. Uemura T, Takamatsu K, Ikeda M, Okada M, Kazuki K, *et al.* (2012) Transplantation of induced pluripotent stem cell-derived neurospheres for peripheral nerve repair. Biochemical and Biophysical Research Communications 419(2): 130-135.

24. Edstrom A, Briggman M, Ekstrom PA (1996) Phospholipase A_2 activity is required for regeneration of sensory axons in cultured adult sciatic nerves. Journal of Neuroscience Research 43(2): 183-189.

25. Kogteva GS, Bezuglov VV (1998) Unsaturated fatty acids as endogenous bioregulators. Biochemistry 63(1): 6-15.

26. Iwanicki JK, Lu W, Taeusch HW (2010) Reductions of phospholipase A(2) inhibition of pulmonary surfactant with hyaluronan. Experimental Lung Research 36(3): 167-174.

27. Nitzan DW (2001). The role of hyaluronic acid in protecting surface-active phospholipids from lysis by exogenous phospholipase A(2). Rhematology (Oxford) 40(3): 336-340.

28. Novikov SM, Maksimov GV, Volkov VV, Shalygin AN (2008) Study of the influence of weak constant magnetic field on the excitability of nerve cells. Biophysics 53(3): 519-523.

29. Morisaki S, Oda R, Ota C, Kubo T, Matsuda K-I, et al. (2013) Application of Raman spectroscopy for visualizing biochemical changes during peripheral nerve injury *in vitro* and *in vivo*. Journal of Biomedical Optics 18(11): 116011.

30. Li R, Slipchenko MN, Wang P, Cheng J-X (2013) Compact high power barium nitrite crystal-based Raman laser at 1197 nm for photoacoustic imaging of fat. Journal of Biomedical Optics 18(4): 040502.

Citation: Isakina MV, Revina NV, Revin VV (2015) Influence of Potassium Hyaluronate on the Content of Lysophospholipids and Free Fatty Acids in Damaged Somatic Nerves of Rat. Biol Med (Aligarh) 7(2): BM-071-15, 4 pages.