# Changes in Phospholipid Composition of the Spinal Cord in Rabbits with Allergic Encephalomyelitis as an Experimental Model of Multiple Sclerosis

**E. S. Revina, N. V. Gromova, and T. E. Timoshina**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 152, No.8, pp. 184-187, August, 2011
Original article submitted June 23, 2010

Qualitative and quantitative composition of phospholipids in different compartments of the spinal cord was studied in normal rabbits and in experimental model of multiple sclerosis. The content of initial and final LPO products in compartments of the rabbit spinal cord was studied during different stages of the disease. The emergence of quantitative changes in the phospholipid composition depended on the form of experimental allergic encephalomyelitis and compartment of the spinal cord. The levels of conjugated dienes and MDA increased. The maximum levels of LPO products were recorded in the lumbar and sacral compartments of the spinal cord in severe experimental allergic encephalomyelitis.

**Key Words:** *multiple sclerosis; experimental allergic encephalomyelitis; myelin; phospholipids; lipid peroxidation*

Study of demyelinating diseases, *e.g.* multiple sclerosis, is a pressing problem of modern biology and medicine. Multiple sclerosis involves multifocal destruction of myelin, which leads to nerve pulse conduction disorders. Some regularities in the pathomorphological picture of demyelinization have been revealed and the neurological symptoms of the disease, developing as a result of myelin degradation, have been characterized [4,7]. One of little studied problems is the disorders of lipid homeostasis in multiple sclerosis. Experimental allergic encephalomyelitis (EAE), an autoimmune disease similar to multiple sclerosis by clinical manifestations and histological signs, is the most adequate model of multiple sclerosis [5].

We studied qualitative and quantitative composition of phospholipids (PL) and the intensity of LPO processes in various compartments of the spinal cord in rabbits in health and EAE.

Since lipids are the main components of the myelin membranes, the data on their changes in disease are essential for comprehensive understanding of the demyelination process.

N. P. Ogarev Mordovian State University, Saransk, Russia. ***Address for correspondence:*** nataly_grom@mail.ru. N. V. Gromova

## MATERIALS AND METHODS

Rabbit spinal cord and the lipids isolated from its compartments were studied. The study was carried out on adult male rabbits (2.5-3.0 kg), in which EAE was induced by a single intracutaneous injection in the paw pads of 1 ml encephalitogenic emulsion (0.25 ml per paw) containing 20 mg lyophilized rabbit myelin from the spinal cord, 0.2 ml saline, and 0.8 ml complete Freund adjuvant (100 mg heat-inactivated dry *M. tuberculosis*, 15 mg lanolin, and 15 g mineral oil oil). The first symptoms of EAE manifested after the latent period of 10-12 days: the general status and appetite deteriorated, the animals lost weight, and by the end of week 2 the first neurological symptoms manifested, which later progressed, reaching the maximum on days 23-27. The animals developed motor disorders and dysfunctions of the pelvic organs.

The animals were sacrificed by air embolism at the peak of EAE symptoms (usually on day 27), and

0007-4888/11/1522-0224 

4 spinal compartments (cervical, thoracic, lumbar, sacral) were examined. The lipids were then extracted with chloroform and methanol mixture, after which PL were separated by thin layer chromatography in silica gel. Individual PL were measured by means of a calibration curve plotted using standard samples containing inorganic phosphate (0-10 μg $KH_2PO_4$) as described previously [10].

The levels of conjugated dienes in the lipids and MDA were measured by spectrophotometry with TBA as described previously [2]. The data were statistically processed by methods of variation statistics using Student's *t* test.

## RESULTS

Normally, qualitative composition of PL from rabbit spinal cord includes six fractions: lisophosphatidylcholine, sphingomyelin, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, and phosphatidylethanolamine (Table 1).

Injection of encephalitogenic emulsion induced changes in quantitative composition of PL (Table 2). PL sum reduced in comparison with the control in all cases, more intensely in the lumbar and sacral compartments (Tables 1, 2).

This confirmed the hypothesis according to which initiation of EAE mediated by PL destruction caused demyelinization processes [3].

The percentage of individual PL in the spinal cord changed in rabbits with EAE, particularly with the severe condition. Sphingomyelin level in the cervical spinal cord increased by 17.5% in mild EAE, by 42.1% in the medium-severe form, and by 47.5% in severe disease in comparison with the control. The percentage of other fractions reduced, maximally in severe EAE (Table 2).

Changes in the percentage of individual PL in the thoracic spinal cord were similar to changes in the cervical portion (Tables 1, 2).

A 12.4% increase of sphingomyelin level and decrease of phosphatidylcholine and phosphatidylethanolamine levels by 6.7 and 3.4%, respectively, in comparison with the control were found in the lumbar portion of the spinal cord in mild EAE. In medium severe EAE, the level of sphingomyelin increased by 15.5%, while the levels of phosphatidylcholine and phosphatidylethanolamine decreased by 14.1 and 5.4%, respectively, in comparison with the control. In severe EAE, sphingomyelin level increased by 28.4%, while the levels of phosphatidylcholine and phosphatidylethanolamine decreased by 29.8 and 13.8%, respectively. The level of phosphatidylserine and phosphatidylinositol decreased maximally by 14 and 73.5%, respectively, in comparison with the control.

In the sacral spinal cord, the level of sphingomyelin increased by 6.5% in mild EAE, by 10.6% in medium severe EAE, and by 28% in severe EAR (Tables 1, 2). Other PL fractions decreased in mild EAE and maximally decreased in severe EAE (Table 2). Hence, changes in the phospholipid composition in EAE were similar in all compartments of the spinal cord.

Phosphatidylcholine lisoforms appeared and their levels increased in all compartments of the rabbit spinal cord. Maximum elevation (by 11%) thereof was found in the lumbar portion of the spinal cord in severe EAE. This was due to stimulation of phospholipase $A_2$ hydrolyzing ester bond in position 2 of PL with the formation of free fatty acids [9].

Increase of sphingomyelin percentage was presumably associated with the deficiency of acid sphingomyelinase (enzyme needed for sphingomyelin hydrolysis into ceramide and phosphorylcholine), observed in cases with neurodegenerative phenotype. According to some data, the increase of sphingomyelin content was associated with reduction of the nerve pulse conduction [11].

Decrease of the choline pool in the membrane in some demyelinating diseases increases significantly

**TABLE 1.** Phospholipid Composition of the Rabbit Spinal Cord in Health, $P_n$ μg/TL mg (*M*±*m*)

| PL | Spinal cord compartment | | | |
|---|---|---|---|---|
| | cervical | thoracic | lumbar | sacral |
| Sphingomyelin | 0.183±0.001 | 0.194±0.002 | 0.194±0.001 | 0.293±0.005 |
| Phosphatidylcholine | 0.279±0.006 | 0.310±0.021 | 0.255±0.002 | 0.269±0.006 |
| Phosphatidylserine | 0.171±0.002 | 0.165±0.003 | 0.164±0.002 | 0.210±0.003 |
| Phosphatidylinositol | 0.081±0.002 | 0.060±0.001 | 0.068±0.002 | 0.047±0.004 |
| Phosphatidylethanolamine | 0.475±0.004 | 0.410±0.013 | 0.354±0.002 | 0.456±0.005 |
| PL sum | 1.189 | 1.141 | 1.035 | 1.275 |

**TABLE 2.** Phospholipid Composition of the Rabbit Spinal Cord in Various Forms of EAE, $P_n$ μg/TL mg (*M*±*m*)

| PL | Spinal cord compartment | | | |
|---|---|---|---|---|
| | cervical | thoracic | lumbar | sacral |
| Mild EAE | | | | |
| Sphingomyelin | 0.215±0.006* | 0.226±0.001* | 0.218±0.002* | 0.312±0.001* |
| Phosphatidylcholine | 0.265±0.005* | 0.295±0.001* | 0.238±0.004 | 0.239±0.004* |
| Phosphatidylserine | 0.168±0.004* | 0.152±0.001* | 0.158±0.065* | 0.192±0.002* |
| Phosphatidylinositol | 0.064±0.001* | 0.043±0.011 | 0.035±0.001* | 0.035±0.001 |
| Phosphatidylethanolamine | 0.418±0.017 | 0.381±0.001* | 0.342±0.001* | 0.409±0.001* |
| PL sum | 1.13 | 1.097 | 0.99 | 1.187 |
| Medium-severe EAE | | | | |
| Sphingomyelin | 0.260±0.006* | 0.251±0.008* | 0.224±0.002* | 0.324±0.008* |
| Phosphatidylcholine | 0.244±0.007* | 0.276±0.003* | 0.219±0.007* | 0.233±0.012 |
| Phosphatidylserine | 0.160±0.002* | 0.146±0.002* | 0.148±0.004* | 0.178±0.004* |
| Phosphatidylinositol | 0.054±0.003* | 0.033±0.002* | 0.027±0.004* | 0.027±0.002* |
| Phosphatidylethanolamine | 0.407±0.002* | 0.351±0.006* | 0.335±0.008* | 0.384±0.008* |
| PL sum | 1.125 | 1.057 | 0.953 | 1.146 |
| Severe EAE | | | | |
| Sphingomyelin | 0.270±0.006* | 0.260±0.005 | 0.249±0.002* | 0.375±0.003* |
| Phosphatidylcholine | 0.220±0.008 | 0.247±0.003* | 0.179±0.002* | 0.192±0.005 |
| Phosphatidylserine | 0.152±0.001* | 0.143±0.011 | 0.141±0.001* | 0.163±0.003* |
| Phosphatidylinositol | 0.035±0.004 | 0.028±0.005 | 0.018±0.001 | 0.016±0.001* |
| Phosphatidylethanolamine | 0.334±0.005* | 0.317±0.001* | 0.305±0.012 | 0.346±0.008* |
| PL sum | 1.01 | 0.995 | 0.892 | 1.092 |

**Note.** *$p<0.05$ in comparison with the control.

the acetylcholine release, as a result of which the neuronal reserves of choline-containing PL are exhausted, cell vulnerability increases, and cell death is stimulated [1]. Initiation of EAE was associated with a decrease of phosphatidylcholine content in comparison with the control.

Phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol are oxidized most easily and are highly unsaturated, and hence, decrease of their levels was presumably due to involvement of LPO in the demyelination process.

Experimental allergic encephalomyelitis stimulates phospholipases C cleaving off the phosphatide acid residue with amino alcohol primarily the phosphatidylinositol-specific phospholipase C hydrolyzing phosphatidylinositol; this causes loosening of the membrane and facilitates the access for other phospholipases cleaving off fatty acids [8].

Changes in the levels of the primary and final LPO products depend on the EAE form. The maximum accumulation of conjugated dienes is found in the lumbar and sacral portions of the spinal cord (3.5 and 5 times, respectively) in severe EAE. Analysis of TBA-active products in EAE showed a significant increase of MDA in the lumbar (2.5 times) and sacral (3.3 times) portions, this indicating accumulation of LPO products.

Hence, the greatest changes in the quantitative composition of PL were found in severe EAE, particularly in the lumbar and sacral portions of the spinal cord. A significant increase in the levels of the first and final LPO products was also found in these compartments of the spinal cord. These data indicate the involvement of the lipid phase of nerve structures in the pathogenesis. This is in line with the hypothesis on the important role of LPO in demyelination processes in multiple sclerosis.

## REFERENCES

1. E. M. Vasilyeva and M. I. Bakanov, *Biomed. Khim.*, **51**, No. 6, 581-602 (2005).

2. Yu. A. Vladimirov and I. M. Archakov, *Lipid Peroxidation in Membranes* [in Russian], Moscow (1972), P. 241-242.
3. E. I. Gusev, T. L. Demina, and A. N. Boiko, *Multiple Sclerosis* [in Russian], Moscow (1997).
4. I. A. Zavalishin and V. Ch. Golovina, *Multiple Sclerosis. Selected Problems in Theory and Practice* [in Russian], Moscow (2000).
5. T. A. Zargarova and O. O. Favorova, *Immunologiya*, No. 2, 5-8 (1999).
6. I. D. Stolyarov, A. M. Petrov, and A. G. Il'ves, *Allergol. Immunol.*, No. 3, 370-372 (2009).
7. T. E. Schmidt and N. N. Yakhno, *Multiple Sclerosis* [in Russian], Moscow (2003).
8. A. Bosio, H. Bussow, J. Adam, and W. Stoffel, *Cell Tissue Res.*, **292**, No. 2, 199-210 (1998).
9. A. Kalyvas and S. David, *Neuron*, **41**, No. 3, 323-335 (2004).
10. V. E. Vaskovsky, E. V. Kostetsky, and J. Vasendin, *J. Chromatogr.*, **144**, 129-141 (1975).
11. D. D. Wood and M. A. Moscarello, *Molecular Biology of Multiple Sclerosis*, Ed. W. C. Russel, New York, Toronto (1997), pp. 38-54.