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Myelin composition of spinal cord in a model of amyotrophic lateral sclerosis (ALS) in SOD1^{G93A} transgenic rats

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Abstract

We present the results of biochemical and electron microscopic (EM) examinations of the spinal cord myelin from SOD1^{G93A} transgenic Sprague Dawley rats in the early and late symptom-free period of the disease (60 and 93 days of life) and after four-leg paralysis has occurred (120 days of life). Biochemical and ultrastructural changes of myelin started already in the symptom-free period and become most pronounced in the paralyzed animals. Biochemical examinations indicated a decrease of lipids, phospholipids, cholesterol and cerebrosides. The pattern of particular phospholipids was in the normal range. A progressive decrease of the percentages of proteolipid, DM-20 and Wolfgram proteins was evident. Myelin basic proteins I and II were less affected. In EM, massive myelin disorganization was observed.

Key words: *amyotrophic lateral sclerosis model, myelin composition*

Introduction

Amyotrophic lateral sclerosis (ALS) is not a demyelinating disease and the pathological processes do not directly attack myelin sheaths that surround and protect nerve fibres of the brain and spinal cord. Probably for this reason little attention has been paid to the changes in myelin structure and composition in this disease. A notable exception is a recent paper of Hayashi et al. [14] showing that in the majority of ALS patients diffuse myelin pallor in the anterolateral columns beyond the corticospinal tracts with various degrees of diffuse macrophage infiltration occurs, which suggests that it may be derived from intrinsic spinal cord lesions.

Transgenic rodents expressing mutated human SOD1 forms found in the familial type of ALS (most frequently the G93A mutation) develop neuromuscular disease similar in many aspects to human ALS [13,17,22,26].

The aim of this study was to describe some electron microscopic (EM) and biochemical features of spinal cord myelin of SOD1^{G93A} transgenic rats of various age and stage of disease progression.

Material and Methods

The material comprised asymptomatic 60-day-old (n=3), asymptomatic 93-day-old (n= 3) and symp-

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tomatic with four-leg paralysis 120-day-old (n=3) $SOD1^{G93A}$ Sprague-Dawley albino transgenic rats from the colony kept in the Mossakowski Medical Research Institute under the agreement with Wyeth Co. (described in detail in the preceding communication) [15]. Non-transgenic age-matched Sprague-Dawley rats were examined as controls (n=3 of each 60, 93 and 120 days old).

For EM examination a lumbosacral section of the spinal cord was taken and fixed in 5% glutaraldehyde followed by 1% osmium tetroxide (both in cacodylate buffer) for 2 h at 4°C. After dehydration in increasing concentrations of alcohol and acetone, the material was embedded in Epon 812, sectioned in LKB III ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a JEM II electron microscope.

For biochemical investigations myelin was isolated from the spinal cord by the method of Agrawal et al. [1] in a Beckmann ultracentrifuge at 75,000 × g for 60 min. in a discontinuous sucrose gradient. The separated myelin was washed in distilled water and sedimented after centrifugation at 100,000 × g. Lipids were extracted by the method of Folch et al. [11]. Total lipids were determined by the method of Skipski and Barclay [27]. Total phospholipids were determined by Bartlett's method [5]. They were further developed in chloroform:methanol:water (65:30:5) on microscopic plates coated with Silica Gel H (Merck), using one-dimensional thin-layer chromatography, according to the micromethod of Muller and Vahar-Matiar [21]. The chromatograms were developed in iodine vapours. The percentage composition of phospholipids was determined by densitometry in a densitometer GS-700 (Bio-Rad), and MultiAnalyst/PC version 1.1. For the phospholipids identification the Rf values were compared with those of standards (Sigma). Cholesterol was determined directly [32]. Cerebrosides were estimated by the procedure of Hess & Lewin [16]. Protein content was estimated by the method of Peterson [23]. The proteins were separated in the Mini-Protean II system (Bio-Rad) on 4-15% gradient gels according to Laemmli [19], stained with Coomassie Brilliant Blue dye, and further processed in the GelDoc 1000/2000 system (Bio-Rad), imaging densitometer GS-700 (Bio-Rad) Multi-Analyst/PC version 1.1, Quantity One version 4 and Mitsubishi Video Printer P91. Molecular weight standards of proteins (Bio-Rad) were run at the same time on one lane of the polyacrylamide gel.

Statistical analysis

The variances and significance analysis by Wilcoxon's and Student's test were calculated.

Results

In the electron microscopic examination abnormalities of axonal myelin sheath were observed in numerous large axons in the late pre-symptomatic stage (age 93 days of life). In comparison with age-matched control rats (Fig. 1A) the alterations in the myelin structure were represented by areas in which the normal compact appearance of myelin was lost. The myelin lamellae exhibited detachment, separation, dissolution and vacuolization (Fig. 1B). More prominent destruction of the myelin sheath was found in the fully symptomatic stage (age 120 days). Degenerated swelling or dark axons displayed myelin breakdown (Fig. 2B). In some axons myelin debris was still observed around the degenerated axons.

Biochemical examinations of the myelin structural components revealed a progressive loss of total lipid content, starting already in the symptom-free stage (Table I). The most prominent was the decrease in the content of cholesterol and cerebrosides, the former being advanced already in the early presymptomatic stage, the latter being clearly accelerated in the paralyzed animals. The total content of phospholipids decreased along with disease progression; their composition, however, did not change. Decreases in the myelin protein composition were apparent already in the symptom-free period, and they progressed afterwards. Wolfgram's, proteolipid and the DM-20 proteins appeared to be affected more than myelin basic proteins I and II (Fig. 3, Table II).

Discussion

In the experimental model of amyotrophic lateral sclerosis in rats and mice and in human ALS a progressive loss of motoneuron cells within the anterior horns of the spinal cord is the main abnormality. The nerve cell death leads to axonal degeneration, which affects both the axons and the myelin sheath [10,12,25].

The two main components of the myelin sheath are lipids and proteins. Lipids account for 70 to 80% of the myelin components. The major lipids of myelin are represented by cholesterol, which is the

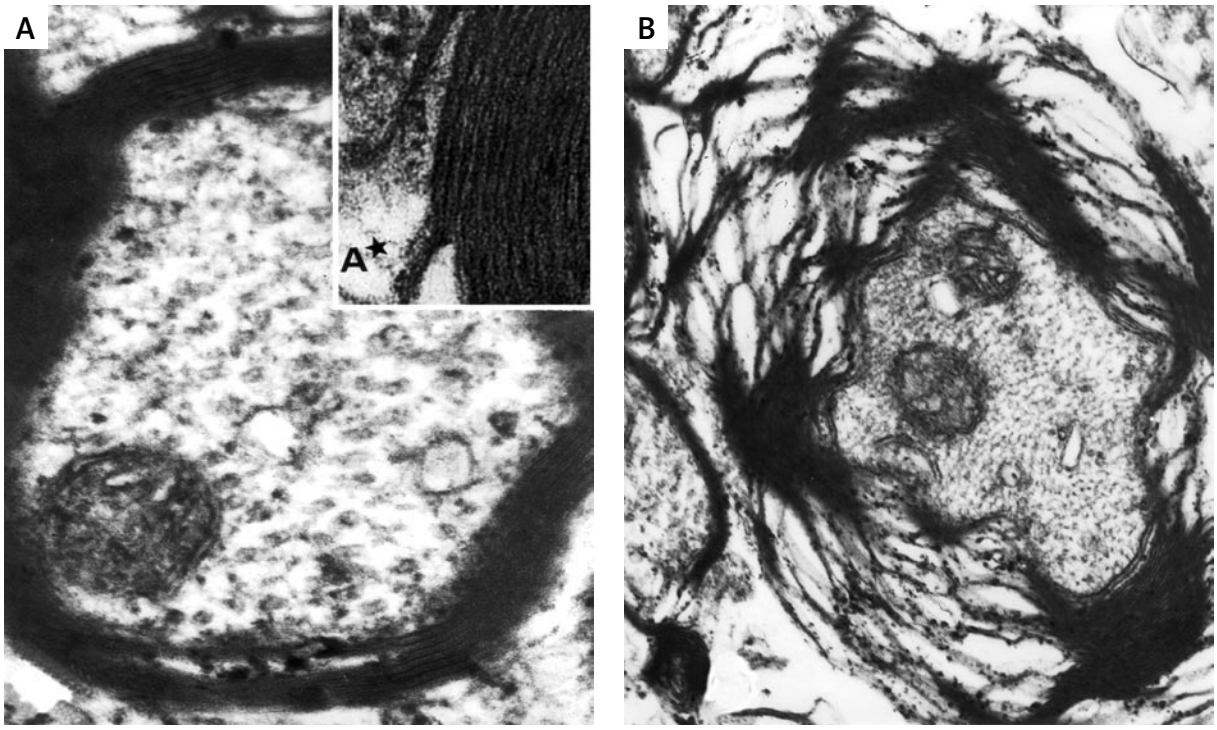

Fig. 1. Electron micrograph of spinal cord of transgenic SOD1 rat. (A) In control rat (90 days old) large axon with preserved myelin sheath. × 50 000. (A*) Well preserved arrangement of myelin lamellae is visible at higher magnification. × 180 000. (B). Early stage of axon degeneration with dissolution and detachment of myelin lamellae in 90-day-old transgenic rat. × 50 000

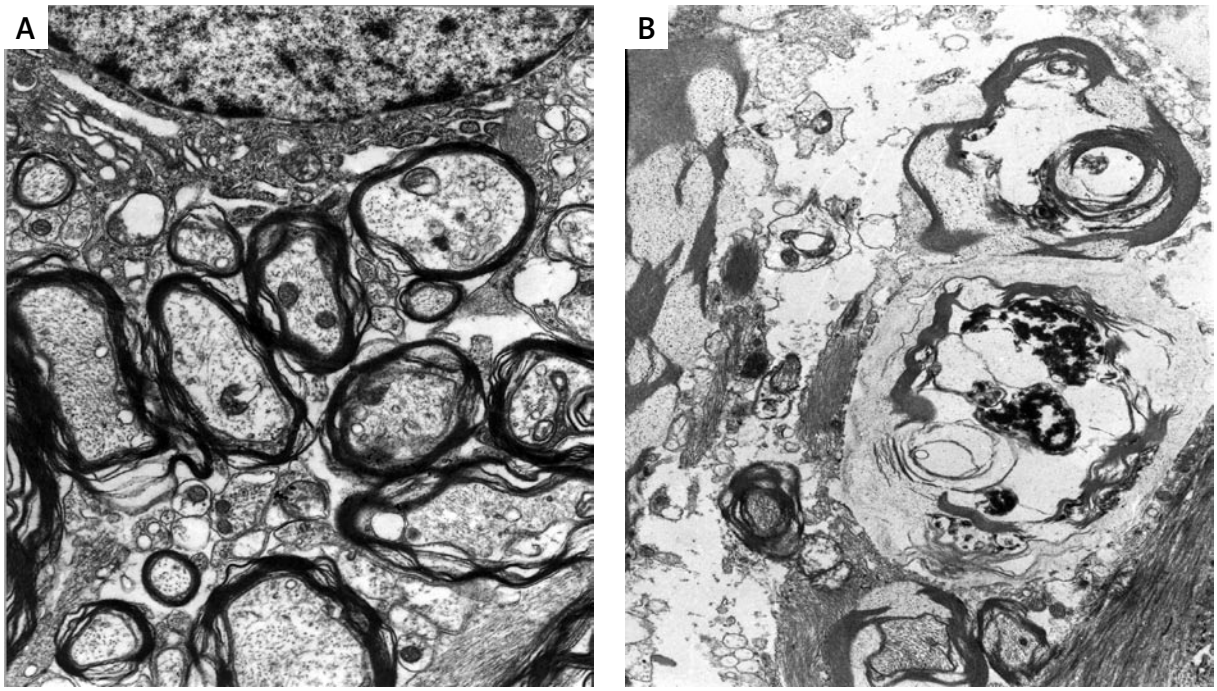

Fig. 2. Electron micrograph of spinal cord of transgenic rats. (A) In control rat (120 days old) numerous normal myelinated axons. (B) Degenerated axons with myelin remnants in 120-day-old transgenic rat. × 20 000

Table I. The ratio of lipid components of spinal cord myelin in transgenic rats and age-matched controls

Component	60 days of life symptoms free n=3	90 days of life symptoms free n=3	120 days of life paralyzed n=3
lipids	0.73±0.10[c]	0.66±0.10[b]	0.54±0.10[b]
phospholipids	0.61±0.10[b]	0.45±0.08[a]	0.44±0.06[a]
phosphatidylcholine*	0.99±0.01	0.97±0.03	0.89±0.11
phosphatidyl ethanolamine*	0.99±0.10	1.04±0.04	0.97±0.08
sphingomyelin*	0.96±0.06	0.99±0.03	0.91±0.10
cholesterol	0.42±0.12[b]	0.33±0.07[a]	0.15±0.06[a]
cerebrosides	0.90±0.10	0.81±0.10[d]	0.33±0.10[a]

*The values represent means ± SD of the ratio of particular lipids (in µg per mg of myelin proteins) in 3 normal animals of each age (the mean values = 1.0) and the transgenic rats. * These values represent means ± SD of the ratio between the percentage of particular phospholipids separated by thin-layer chromatography in 3 normal animals of each age (the mean values = 1.0) and transgenic rats. [a] p<0.001, [b] p<0.005, [c] p<0.01, [d] p<0.05*

Table II. The ratio of protein components of spinal cord myelin in transgenic rats and age-matched controls

Component	60 days of life symptoms free n=3	90 days of life symptoms free n=3	120 days of life paralyzed n=3
Wolfgram's protein	0.60±0.10[c]	0.50±0.10[c]	0.32±0.10[a]
proteolipid protein	0.61±0.10[c]	0.59±0.10[a]	0.56±0.10[a]
DM-20 protein	0.70±0.09[b]	0.48±0.11[c]	0.33±0.10[b]
myelin basic protein I (18 kD)	0.83±0.06[b]	0.64±0 07[a]	0.70±0.08[a]
myelin basic protein II (16 kD)	0.85±0.10	0.65±0.05[a]	0.53±0.06[a]

The values represent means ± SD of the ratio of the percentage of particular proteins separated using PAGE electrophoresis in 3 normal animals of each age (the mean values = 1.0) and transgenic rats. [a] p<0.001, [b] p<0.005, [c] p<0.01

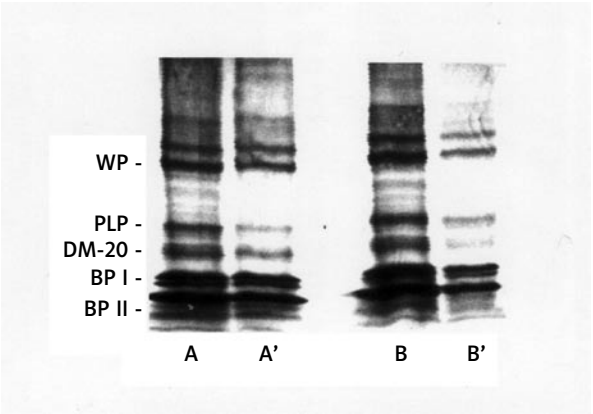

Fig. 3. Profile of myelin of spinal cord proteins in transgenic SOD1 mutant rats. (A) In a normal rat, 60 days old, (A') in a symptom-free transgenic rat, 60 days old, (B) in a normal rat, 120 days old, (B') in a paralyzed rat, 120 days old. WP – Wolfgram's protein, PL – proteolipid protein, DM-20 protein, BP – basic proteins I and II

major architectural component of compact myelin and accounts for 20-25% of the total lipids. Cholesterol determines the permeability and fluidity of the myelin membranes. Among myelin proteins the proteolipid protein (PLP) is most abundant and has multiple functions. Among these functions there is membrane adhesion and compaction of myelin, interactions of oligodendrocytes with axons, maintenance and wrapping of axons, and development of oligodendrocytes. Lipids and proteins work synergistically to provide adhesion and the right membrane structure. Changes in the structural elements lead to destabilization of this membrane, which disorganize both their synthesis and degradation. Despite tremendous efforts in molecular biology the processes of myelin synthesis and its breakdown, even in physiological conditions, is largely unknown. Understanding these processes may be of primary importance for understanding several neurological diseases, including ALS.

In ALS, as well as in mutant SOD1 transgenic rodents, considered to be models of the familial form of this disease, a progressive loss of motoneurons within the anterior horns of the spinal cord is the main abnormality. Whereas most authors seem to believe that death of motoneuronal somata is a primary event, which is followed by secondary axonal degeneration affecting both the axons and the myelin sheath, other possibilities cannot be excluded. The rates of cholesterol synthesis and cholesterol concentration in the murine spinal cord tissues are higher by five and two times, respectively, than in the cerebrum and cerebellum [24]. Recently Anderson et al. [2] showed that mice inactivation of liver X receptor β, which may be involved in cholesterol trafficking between tissues, produces adult-onset motoneuron degeneration. These authors suggested that pathological neurotoxic accumulation of sterols and lipids may be involved in chronic motoneuron diseases, such as ALS.

No data are available yet on the myelin structure and function of myelin either in amyotrophic lateral sclerosis or in its model in transgenic mice and rats.

The presented data indicate that in the spinal cord myelin of $SOD1^{G93A}$ transgenic rats marked deviations in lipid composition occurred. The most pronounced decrease concerned cholesterol, which appeared to decrease substantially faster and significantly more than the other myelin components. The decrease of cholesterol, evident already in symptom-free animals, may weaken the molecular structure of the myelin membrane and play a significant role in the early pathomechanism of this disease. Degradation of the main myelin proteins, although it occurs later, may also be essential in destabilization of the myelin membrane. The observed changes in the myelin composition are likely to alter its fluidity, membrane curvature and vesiculation.

The mechanism(s) responsible for myelin changes in the spinal cord of $SOD1^{G93A}$ transgenic rats remain unknown. Since ALS is not a demyelinating disease, changes in myelin content are likely to be secondary to degeneration of motoneuron somata. If the same holds true for the transgenic model of ALS, one may expect that changes in spinal cord myelin which occur in these animals may, at least to some extent, resemble those which occur during Wallerian degeneration. A model of CNS Wallerian degeneration is the degeneration of axons dissected or crushed optic nerve, which possesses features of central white matter tracts. The biochemical basis of Wallerian degeneration of the optic nerve has been studied in detail [30,31] and it was found that the earliest and the most prominent feature of this process is the decrease of cholesterol due to its conversion to cholesterol esters, whereas changes in the myelin proteins (Wolfgram's protein, MBP) appeared to be more delayed. Moreover, in those studies phosphatidylcholine appeared to be the lipid species most resistant to degradation. It should also be noted that the vast majority of the debris removed during Wallerian degeneration of rat optic nerve was found to be removed by marcophages [20,28], which were also found to infiltrate the brain stem and spinal cord in axonal degeneration in the majority of ALS patients [14].

In the present study evident changes in spinal cord myelin composition are observed, and they are, at least qualitatively, similar to those which occur in the optic nerve during CNS Wallerian degeneration. Nevertheless, the other possibility, which should also be taken into account, is that changes of myelin are causally related to dysfunction of mitochondria in axons and in the adherent cells. Although compact myelin is devoid of mitochondria, these organelles are present in Schmidt-Lantermann oligodendroglial cytoplasmic clefts [4,7,9]. Mitochondria are also present in cytoplasmic islands between paramodal myelin loops. Mitochondrial damage is a prominent feature of ALS as well as its transgenic models. It is seen as dilated and disorganized cristae, leakage and interruption of the outer membrane and early vacuoles, and it precedes the onset of clinical symptoms [10,18]. Although lipid peroxidation, as well as oxidative changes of proteins and oxidative damage to mitochondrial DNA, has been observed [29], mitochondrial damage seems to be a central process in the degeneration mechanisms. Degeneration of the axons running in parallel may thus be the result of mitochondrial damage, as the mitochondria are the most prominent source of oxidative free radicals [6]. Apart from mitochondria, mutations of SOD1 can also lead to increased production of "hydroxyl radical-like" activity, which further provides oxidative damage to macromolecules [8]. It is also implicated that increased hydroxyl radical production associated with SOD1 mutation and/or lipid peroxidation-derived radicals causes protein oxidative damage and the SOD1 itself is a target which may compromise its antioxidant action [3].

In conclusion, in SOD1^{G93A} rats the electron microscopic and biochemical studies indicate early changes in spinal cord myelin, which are qualitatively similar to those of Wallerian degeneration of the optic nerve. The possibility that myelin changes in the ALS transgenic model are related to the dysfunction of mitochondria in axons and the adherent cells cannot be excluded.

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