Fine Structural Observations of Neurofilamentous Changes in Amyotrophic Lateral Sclerosis

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Abstract. Twenty-two of 32 sporadic cases of amyotrophic lateral sclerosis had argyrophilic spheroids, 20 micrometers or larger, in the anterior horns of the spinal cords. The fine structure of these spherical bodies was characterized by interwoven, small bundles of 10 nm neurofilaments. Scattered mitochondria, vesicles and fragments of smooth endoplasmic reticulum were commonly found among the bundles of neurofilaments. The spheroids were present not only in the myelinated axons, but also in the perikarya of the anterior horn cells. In anterior horn neurons occasional fragments of rough endoplasmic reticulum, lipofuscin and even nuclei were found among the neurofilaments, in addition to the other components. Rarely, some filamentous accumulations contained unusual features such as paracrystalline arrays, polyglucosan bodies and honeycomb-like structures. Linear densities, associated with ribosome-like particles, were found scattered within focal collections of randomly arranged neurofilaments in some perikarya of two cases. Occasional mitochondria with regularly arranged short protrusions on the outer membrane were observed in the myelinated axons in one case.

Key Words: Amyotrophic lateral sclerosis; Anterior horn neurons; Axonal swelling; Neurofilaments.

INTRODUCTION

The abnormal accumulation of 10 nm neurofilaments in anterior horn neurons is a noteworthy finding in amyotrophic lateral sclerosis (ALS) for two reasons: 1) it may represent an early morphological change in ALS (1–3); 2) the accumulation of neurofilaments constitutes the main characteristic pathological feature in several animal systems which have been suggested as models for motor neuron diseases (4–6), and it is important to compare these models with human ALS. This report is an analysis of the fine structure of the neurofilamentous accumulation in large anterior horn neurons in 22 cases of sporadic ALS, along with a review of previous studies on neurofibrillary changes in ALS.

MATERIALS AND METHODS

Between 1974 and 1983, 32 cases of sporadic ALS were verified at autopsy at St. Vincent’s Hospital, New York. Postmortem examinations were performed within two to six hours (h) of death. Tissue blocks were obtained from the cervical (30 cases) and lumbosacral (29 cases) levels of the spinal cord for light microscopic study. These blocks were fixed in formalin and

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Supported by NIH Grant #2 P50 NS11605-08. Presented in part at the 59th Annual Meeting of the American Association of Neuropathologists, St. Louis, MO, June 9–12, 1983.
Fig. 1. Spheroids in the anterior horn of the lumbar cord. A. H & E. × 350. B. Bielschowsky Silver impregnation. × 700.

Fig. 2. Increased number of 10 nm neurofilaments in the perikaryon of an anterior horn cell in ALS. × 38,000.

embedded in paraffin. Cross-sections of these blocks were cut at 6 μm for hematoxylin and eosin (H & E) stain and at 8 μm for silver impregnations. Two to three sections were examined at each level.

For electron microscopic examination, additional tissue blocks were obtained from the adjacent anterior horns of the cervical cord (19 cases) and lumbosacral cord (22 cases). Approximately 2 mm thin cross-sections were cut and the anterior half of the gray matter was trimmed and further minced into small blocks. The number of blocks at each level embedded
was approximately 15. These were immersed in 2.5% glutaraldehyde in 1/15 M phosphate buffer, pH 7.4, post-fixed with 1% osmium tetroxide for one h, dehydrated, and embedded in Epon. In addition, cervical (two cases) and lumbar (five cases) anterior roots, as well as portions of sciatic nerves (two cases), were similarly prepared.

Levels for further study were selected on the basis of the presence of spheroids 20 µm or larger in diameter. This criterion was used in order to exclude the similarly staining, but substantially smaller “globules” seen even in normal elderly individuals (1). Semi-thick sections from the corresponding levels were cut from the Epon block and stained with toluidine blue. After identification of spheroids or other unusual structures the block was further trimmed and thin sections were cut, stained with uranyl acetate and lead citrate and examined with the electron microscope.

RESULTS

Light Microscopy

The histological characteristics of these cases of sporadic ALS have been previously described (7). In ten cases no spheroids were seen. Of the remaining 22, eight showed spheroids in both the cervical and lumbosacral cords. Two others showed them in only the cervical cord (in one case, the lumbar cord was not available) and in 12 others only the lumbosacral cord contained spheroids (in one of these cases, the cervical cord was not available).

The frequency of spheroids in those cervical cords which contained them was substantially lower than in the lumbosacral cord. Usually only one or two spheroids were seen in a section of the cervical cord (only one case had as many as three) while as many as nine were seen in a section of the lumbosacral cord. In nine of the cases in which spheroids were seen in the lumbosacral cord, three or more were found in a single section, while the others showed only one or two per section. The spheroids (Fig. 1A, B) were argyrophilic and identified as pale, eosinophilic, or sometimes faintly basophilic structures containing a wavy or whorl-like pattern of fine fibrils in H & E stain.

The location of the spheroids was usually within the zones of motor nuclei of the anterior horns, but some were outside the nuclei themselves, near the anterior edges of the anterior horns (7).

Electron Microscopy

Spheroids were identified electron microscopically in two of 19 cases of cervical anterior horn examined and ten of 22 cases of lumbar anterior horn examined. The spheroids consisted of the accumulation of bundles of neurofilaments with characteristic side arms.

The site of filamentous accumulation was easily identifiable as the cell body when perikaryal organelles, such as Nissl substance, fragments of rough endoplasmic reticulum, and lipofuscin granules were present (Fig. 2). At other times the spheroids were surrounded by myelin sheaths and could be placed within axons. However, when the tangles of neurofilaments were massive, their precise location, whether in the cell body or axon, was sometimes difficult to determine. The bundles of neurofilaments in the spheroids were arranged in a characteristic interwoven pattern (Figs. 3, 4). Large bundles of parallel filaments that displaced organelles, either in the perikarya or axon, were exceptional. In addition, the spheroids contained scattered mitochondria, vesicles and fragments of smooth endoplasmic reticulum. Some filamentous accumulations contained unusual features. Paracrystalline arrays (resembling Hirano bodies) were seen within the tangles of neurofilaments in one

Fig. 3. A portion of a spheroid in an anterior horn in ALS. The neurofilamentous bundles show the characteristic interwoven patterns. Mitochondria and vesicles are intermingled with the filamentous bundles. ×12,000.

Fig. 4. Higher magnification of an area similar to that illustrated in Figure 3. The small bundles are composed of 10 nm neurofilaments. ×35,000.

spheroid of one case (Fig. 5). Polyglucosan bodies were observed in ten cases, frequently in axons as well as occasionally in the soma. Usually they were separated from surrounding organelles, including neurofilaments. In five instances the polyglucosan body was observed within the spheroid and these two structures were
Fig. 5. Paracrystalline arrays within a spheroid in ALS. ×44,000.
Fig. 6. Corpus amylaceum within a spheroid in ALS. ×27,000.

immediately adjacent to one another (Fig. 6). Honeycomb-like structures were observed in spheroids from two cases (8). In a third case a honeycomb-like structure was also identified within the soma of a neuron apparently with no associated filamentous accumulation.

Linear densities, associated with ribosome-like particles were found scattered within focal collections of randomly arranged neurofilaments in some perikarya of two cases (Fig. 7).
Fig. 7. Scattered linear densities and granules within a focal accumulation of randomly distributed 10 nm filaments in a chromatolytic anterior horn cell. ×34,000.

Fig. 8. A mitochondrion within a myelinated axon of an anterior root with periodic stubby protrusions at the outer membrane. ×112,000.

In one case, the outer membrane of several mitochondria had periodic stubby protrusions. These protrusions measured approximately 5 nm in greatest diameter (Fig. 8). Such mitochondria were observed occasionally in myelinated axons of spinal roots and peripheral nerves without any apparent abnormalities.
DISCUSSION

Spheroids filled with 10 nm neurofilaments were observed by Carpenter (1) in the proximal axons of anterior horn cells in sporadic ALS. He suggested that the formation of spheroids may be an early histological change in ALS. His observation was subsequently confirmed by others (9–12). More recently, in a case of sporadic ALS with an unusually short clinical course, abundant spheroids in the anterior horns of the spinal cord were observed light microscopically (2). Accumulations of 10 nm neurofilaments were identified electron microscopically, not only in the myelinated axons, but also in the soma of many surviving anterior horn cells (3). The present investigation of the large collection of ALS cases at St. Vincent’s Hospital in New York City (7) revealed that accumulations of 10 nm neurofilaments are seen not only in ALS patients with short clinical courses, but also in the majority of longer term cases, although not in as dramatic a fashion.

Although spheroids are not unusual in ALS there are some cases in which this change is not encountered. Furthermore, the pattern of filamentous accumulation in spheroids is not unique to ALS. Similar accumulations of 10 nm neurofilaments have been observed in a variety of different conditions unrelated to ALS (4–6, 13–17).

Little information is available about the existence of spheroids greater than 20 μm in diameter in the anterior horns of normal spinal cords. Takahashi and Agari (10) studied a case of a 21-year-old control and two cases of subacute myelo-optico-neuropathy (SMON) in addition to four ALS cases. They found no spheroids in the lumbar cord except for one case of SMON in which they described an average of only one per section, although they encountered many argyrophilic “globules” in all of their non-ALS cases. In our study of control non-ALS cases above age 50 at Montefiore Medical Center, one to two spheroids per slide were observed at the lumbar level in ten of 100 cases and at the cervical level in four of 89 cases (7).

There are a number of reports of diseases affecting motor neurons in human patients in which 10 nm filamentous accumulations in the large anterior horn cells constitute the most striking histological findings. In many of these conditions, the pattern of filamentous accumulation is often somewhat different from that described in our sporadic ALS cases. For example, in a case of atypical motor neuron disease, Schochet et al (18) observed the presence of perikaryal filamentous masses with discrete borders which displaced the other organelles, notably the Nissl substance. Similar configurations were reported in a case of multisystem degeneration (19), in macroglobulinemia associated with peripheral neuropathy (20), and in other disorders (21).

An association between other types of filamentous inclusions and the 10 nm neurofilamentous accumulation described here was noted in this study as well as in certain previous publications. Hirano bodies were previously reported in ALS (11, 18), but not in the anterior horns of normal spinal cords. Corpora amylacea within axons are not uncommon, even in the anterior horns in non-ALS elderly individuals, but their close proximity to accumulations of neurofilaments is certainly unusual, although it has recently been described in polyglucosan body disease (22). Honeycomb-like structures within the anterior horn cells have hitherto been reported only in a patient with lathyrism (23). The linear densities associated with ribosome-like granules we observed in focal accumulations of randomly oriented filaments are apparently identical to those ribosome-associated tubular structures reported in a case of sporadic, juvenile ALS by Oda et al in 1978 (24). All these abnormal fibrillary
structures other than the 10 nm neurofilaments are uncommon and their significance remains unknown. Baculovirus, previously reported in ALS (25), was not seen in our material.

The peculiar mitochondrial configuration illustrated in Figure 7 is a puzzling finding. The same configuration was observed by us in an ALS case in the Montefiore Hospital file and also was illustrated in yet another case of ALS by Peña (26). While it may be related to the cytoskeletal filaments connected to mitochondrial outer membranes, as described by Spacek and Lieberman (27), the orderly arrangement of the densities in our material was unlike those patterns illustrated in their report. The periodicity was more reminiscent of the intermembranous structures of neuronal mitochondria reported by Peña as some artifactual product of delayed fixation (28). Precisely similar intermembranous densities, however, were not found in our material. Whether it is an ALS-related phenomenon or a non-specific finding remains to be determined.

The above-described findings in ALS are of special interest because a number of experimental animal models of motor neuron diseases have been described in which accumulation of 10 nm neurofilaments within the anterior horn cell is the main histological feature. Among the effects of certain chemicals (14, 21), major attention has been directed to the results of β-β' iminodipropionitrile (IDPN) intoxication because of a remarkably selective involvement of proximal axons (4, 29). In models of chronic aluminum intoxication, an early appearance of neurofibrillary swelling in proximal axons of neurons was followed by neurofilamentous accumulation in the perikarya and dendrites (15). Increases of 10 nm neurofilaments in the motor neurons has also been described in various other animals. These include zebra, cat, dog, rabbit and pig (6).

Of all the animal models, the accelerated hereditary canine spinal muscular atrophy described by Cork et al (5) is the most reminiscent of human ALS. This condition selectively involves the lower motor neurons and shows chromatolysis in addition to the more prominent neurofilamentous accumulations. While the fine structure of these accumulations are quite reminiscent of those seen in the present study, several important differences must be noted. Firstly, the prominence of the neurofilamentous accumulations in the dog model is far beyond that which we see in the human. Secondly, in the dog model connections between filament-filled processes and the soma are apparently common, whereas they could not be visualized in our study. Carpenter (1) and Hirano (30) and, recently, Kurisaki et al (31) published illustrations of this phenomenon in human ALS. This difference may reflect a significantly different position of the filamentous accumulation in the human axons. Thirdly, polyglucosan bodies, honeycomb-like structures, ribosome-associated linear structures and spiky mitochondria have not been reported in the dog model.

In addition, other differences, not related to the filamentous accumulations, exist between the dog model and human ALS. In typical human ALS, the upper motor neuron system is also involved, unlike in the dog. Human ALS also shows Bunina bodies which have not been described in the dog. Nevertheless, the usefulness of the dog model for an understanding of human ALS must not be discounted. How appropriate it is as a model for human ALS is still to be determined.

ACKNOWLEDGMENT

The authors thank Dr. Herbert M. Dembitzer for his helpful suggestions during preparation of this paper.
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(Received 5 January 1984/Accepted 23 April 1984)
MS84-02