An Autopsy Case of Minamata Disease (Methylmercury Poisoning)—Pathological Viewpoints of Peripheral Nerves

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ABSTRACT

The outbreak of methylmercury poisoning in the geographic areas around Minamata Bay, Kumamoto, Japan in the 1950s has become known as Minamata disease. Based on earlier reports and extensive pathological studies on autopsy cases at the Kumamoto University School of Medicine, destructive lesions in the anterior portion of the calcareous cortex and depletion predominantly of granular cells in the cerebellar cortex came to be recognized as the hallmark and diagnostic yardstick of methylmercury poisoning in humans. As the number of autopsy cases of Minamata disease increased, it became apparent that the cerebral lesion was not restricted to the calcareous cortex but was relatively widespread. Less severe lesions, believed to be responsible for the motor symptoms of Minamata patients, were often found in the precentral, postcentral, and lateral temporal cortices. These patients also frequently presented with signs of sensory neuropathy affecting the distal extremities. Because of few sufficiently comprehensive studies, peripheral nerve degeneration has not been universally accepted as a cause of the sensory disturbances in Minamata patients. The present paper describes both biopsy and autopsy findings of the peripheral nerves in a male fisherman who died at the age of 64 years and showed the characteristic central nervous system lesions of Minamata disease at autopsy. A sural nerve biopsy with electron microscopy performed 1 month prior to his death showed endoneurial fibrosis and regenration myelin sheaths. At autopsy the dorsal roots and eural nerve showed endoneurial fibrosis, loss of nerve fibers, and presence of Büngner’s bands. The spinal cord showed Wallerian degeneration of the fasciculus gracilis (Goll’s tract) with relative preservation of neurons in sensory ganglia. These findings support the contention that there is peripheral nerve degeneration in Minamata patients due to toxic injury from methylmercury.

Keywords: Minamata disease; methylmercury poisoning; human autopsy case; peripheral neuropathy.

INTRODUCTION

It is well known that peripheral neuropathy is a common but nonspecific clinical and histopathological entity. Innumerable toxic agents including organic mercury, as well as vitamin deficiencies, trauma, medications, and many systemic metabolic diseases including diabetes mellitus can produce neuropathy. Except for a few instances in which the primary involvement is with the myelin sheath, the result of injury regardless of the etiological agent is usually similar and relatively stereotyped; namely, axonal degeneration and reactive endoneurial fibrosis.

Though degeneration of peripheral nerves per se is not indicative of organic mercurial intoxication, we consistently observed the degeneration of the peripheral nerves in patients with a well-established diagnosis of Minamata disease from autopsy and biopsy samples at Kumamoto University School of Medicine from 1956 to 1995. These results were documented in the series of publications (1–5, 27–28, 31–36).

Similarly, many investigators have demonstrated the development of neuropathy caused by organic mercury in experimental animals, although there is a wide range of species differences in susceptibility to organic mercury. The peripheral nervous system alone was found to be susceptible to organic mercury in rodents (13–15, 19), while swine (39) and common marmosets (7) showed lesions both in the central and peripheral nervous system similar to those we have seen in human Minamata disease. Degeneration of the myelin sheath in nerves and ganglion cells in the Gasserian ganglion was observed in rhesus monkeys by Hunter et al (9), but peripheral nerve degeneration was not found with either acute and chronic methylmercury intoxication in Macaca mulatta, Macaca fascicularis, or squirrel monkeys (8, 10, 24).

In spite of overwhelming evidence in support of organic mercury inducing peripheral neuropathy in at least in some species, most importantly including humans, some skepticism has remained (12). Our attempt herein is to present an overview of the pathological findings in the central and peripheral nervous system of Minamata disease patients through a case presentation, and to reemphasize the occurrence of peripheral neuropathy in human Minamata disease patients and in common marmosets, which show lesions very similar to those of human patients (6). Whereas methylmercury-treated common marmosets showed lesions in the cerebrum, cerebellum, and peripheral nerves, Macaca mulatta showed lesions from methylmercury poisoning in the cerebrum excluding the cerebellum and no lesions in the peripheral nerves (24–26). Although material from the present human case has been included in previous reports (1, 4, 32–36), representative lesions from the brain and the peripheral nerves are herein presented for comparison with those of the common marmoset (7).
CASE REPORT

A 64-year-old male fisherman who lived in Minamata City in the southern part of Minamata Bay, which was found to be polluted with mercury from the nearby Chisso Factory. Onset of disease was marked by numbness of the feet and disturbance in speech in the spring of 1959. The patient was treated at Minamata City Hospital for pulmonary tuberculosis during the period from May 1965 until July 1968. Neurological examination in October 1968 and December 1969 revealed slight constriction of visual fields on the temporal side, muscular rigidity, increased tendon reflexes, tremor of the fingers, dysgraphia, and adiadochokinesis. Other clinical findings included labyrinthine deafness, hypesthesia, and hypealgesia as well as dysesthesia in the hands and regions below the knees, elevated blood pressure was (170–192 mm Hg), a mask-like face, and dyskinesia. The patient died of massive hemorrhage from gastroduodenal ulcer in January 1970.

AUTOPSY FINDINGS

Autopsy materials from the cerebrum, cerebellum, brain stem, spinal cord, and peripheral nerves were embedded in paraffin and stained with hematoxylin & eosin (H&E) and with the Klüver-Barrera (KB) and Bodian staining methods. Frozen sections were made of peripheral nerves from anterior and posterior root nerve fibers, sciatic nerve, radial nerve, and sural nerve, and stained by the Sugamo myelin (37) and Suzuki’s axon (29) staining methods. The Sugamo myelin stain was modified for use on frozen sections from Kultschizky’s method (11). Inorganic mercury was detected by photoemulsion (23).

The gyri of both hemispheres were atrophic and the sulci were widened. This was particularly remarkable in the calcarine cortex and pre- and postcentral gyri. The surface of the calcarine cortex showed moderate atrophy, and the cortex showed moderate atrophy with widening of the calcarine fissure on the coronal section. Gennari’s band on the calcarine cortex was stained pale with the KB staining method (Figure 1).

Light microscopic observation revealed depopulation of neurons in calcarine, temporal, pre- and postcentral cortices, and the deep white matter showed diffuse degeneration, as is commonly observed in cases of chronic Minamata disease. The calcarine cortex showed severe neuronal loss of whole layers (Figure 2). There was moderate loss of granules cells under the Purkinje cell layer in the cerebellar hemispheres (Figure 3). Mercury granules were detected in Bergman’s glial cells and the granule cell layer using the photoemulsion histochemical method for inorganic mercury (Figure 4). Degeneration of the fasciculus gracilis (Goll’s tract) in the spinal cord was noted, but ganglion cells in the spinal ganglion were relatively well preserved. Sensory nerves, such as posterior roots and sural nerves, were disintegrated, showing Büngner’s bands and a loss of nerve fibers with increase of collagen fibers. Myelinated nerve fibers of the anterior root were well preserved by myelin staining, but myelin sheath destruction was seen in the posterior root. Axon staining showed that axons of anterior root nerve fibers were well preserved (Figure 5), but the posterior nerve fibers showed band-like increases in the small nerve fibers with associated proliferation of fibroblasts and Schwann’s cells (Figure 6).

BIOPSY FINDINGS

As the patient was not initially recognized as having Minamata disease, a sural nerve biopsy was performed on December 9, 1969, about 1 month before his death (1). The tissues were embedded in paraffin and 6-μ sections were stained with H&E. Frozen sections of the nerve fibers fixed in 10% formalin solution were stained with Suzuki’s method (29) for axon and Sugamo method (37) for myelin. A portion of the specimen was immediately fixed in 4% glutaraldehyde solution buffered with 0.05 M pH 7.4 cacodylate buffer for 4 hours. A part of the specimen was then fixed with 1%
osmium tetroxide buffered with 0.1 M 7.4 phosphate buffer, dehydrated, and embedded in epoxy resin using conventional methods to prepare specimens for electron microscopic examination. Epoxy resin (Mercox CL-2B) was purchased from Oken-Shoji Co Ltd (Tokyo, Japan). Sections 1-μm thick were prepared with the LKB microtome, and stained with 0.5% pH 7.4 toluidine blue. Ultrathin sections were double stained with lead and uranyl acetate by Raynold’s method (22), and examined with Hitachi model 11A and 12A electron microscopes.

The biopsy of the sural nerve showed a decrease in myelinated nerve fibers (Figure 7) and increase in small axons with attendant proliferation of fibroblasts and Schwann’s cells (Figure 8). A cross-section of the biopsy of the sural nerve showed a decrease in large nerve fibers and an increase in small nerve fibers and collagen fibers in 1-μ-thick section of epoxy-resin embedded tissue (Figure 9). As a control, Figure 10 shows a cross-section of the sural nerve from a 63-year-old male who died of aortic dissection. The nerve was attached to filter paper and fixed overnight in 2.5% glutaraldehyde. Tangential sections were cut with a laser knife and the specimens were then fixed with 1% osmium tetroxide, embedded in epoxy resin and semithin sections stained with toluidine blue. In addition, ultrathin sections were obtained from the selected areas by Ultracut (Reichert-Jung, Austria) and doubly stained with uranyl acetate and lead citrate for electron microscopy (H-8000, Hitachi, Japan). Large myelinated fibers were well preserved, although small vacuoles and myelin bodies were also observed (Figure 11a). In the areas where unmyelinated fibers were present, the small groups of unmyelinated fibers were seen to be well preserved (Figure 11b). No pathological features indicating axonal degeneration or demyelination were observed.

Electron microscopic observation of the case showed that the morphological changes of the sural nerve included irregular Schwann’s cells, and the appearance of fibroblasts with an increase of collagen fibers (Figure 12). Regressive changes were characterized by degeneration resulting in swollen myelin, wavy degeneration of myelin with extremely

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**Figure 3.**—Granule cells of the cerebellum are decreased in number beneath the Purkinje cell layer. Purkinje cells are partially lost. Klüver-Barrera stain. ×60.

**Figure 4.**—Histochemical demonstration of mercury in the tissue of the cerebellum. Small black granules show inorganic mercury, especially in the Bergman glial cells. ×300.

**Figure 5.**—Axons are well preserved in the anterior root nerve fiber. Suzuki’s axonal stain. ×600.
Figure 6.—Band-like small axons are increased in number with proliferation of fibroblasts and Schwann’s cells in the posterior root nerve fiber. ×600.

Figure 7.—Myelinated nerve destruction and large nerve fibers are decreased in number in the sural nerve. Sugamo myelin stain. ×600.

Figure 8.—Small axons are prominently increased in number with proliferation of fibroblasts and Schwann’s cells in the sural nerve. Suzuki’s axonal stain. ×600.

Figure 9.—A 1-μm cut section of the sural nerve embedded in epoxy resin. The nerve fibers were cut obliquely. Disarrangement and loss of myelinated nerve fibers are seen. The nerve fibers are abnormal in size and collagen fibers are increased in number. Toluidine blue stain. ×600.

thin and electron dense axons (Figure 13), incomplete regeneration including abnormally small axons (Figure 14a, 14b), and incomplete myelination and absence of myelin (Figure 15a, 15b).

Discussion

Although the sensory center in the postcentral cortex is always damaged in Minamata disease, it is difficult to adequately explain the typical signs and symptoms of initial sensory disturbance in the distal extremities by the damage to the sensory center alone. In contrast, the initial lesions in methylmercury poisoning of common marmosets occur in the axons of peripheral nerves (7). It is not clear from acute autopsy cases of Minamata disease whether this is also true in humans, as there have been no precise studies of peripheral nerves (21, 30).
FIGURE 11a.—Electron micrograph of the sural nerve from a control person. Large myelinated nerve fibers are well preserved. The small vacuoles of the myelin sheaths and fine loop structures are considered to be artifacts of the ultrastructural procedures. Bar = 1 μm.

FIGURE 11b.—Electron micrograph of the sural nerve from a control person. Small unmyelinated nerve fibers are arranged in groups and are relatively well preserved. Note that the medial-sized myelinated fibers exhibit normal morphology. Bar = 1 μm.
FIGURE 12.—Electron micrograph of the sural nerve from a patient showing proliferation of processes of Schwann’s cells without axons. Abnormal myelinated fibers are seen (AM). Collagen fibers are remarkably increased in number in the tissue. S = Schwann’s cell, PS = Process of Schwann’s cell. Bar = 1 μm.

FIGURE 13.—Electron micrograph of the sural nerve from the patient showing abnormal wavy regeneration of myelin. The axon is extremely thin and electron dense (arrowheads). Collagen fibers are increased in number surrounding the abnormal nerve fibers. Bar = 1 μm.
FIGURE 14a.—Longitudinal section of the sural nerve biopsy. A small myelinated nerve fiber is found in the increasing collagenous fibers with proliferation of Schwann’s cells formed following damage to the sural nerve. The axons are irregularly small. Bar = 1 \text{ \mu m}.

Axonal change and destruction of the myelin sheath of the sciatic nerves are recognized in severe cases of methylmercury poisoning in the common marmoset (7). However, common marmosets given methylmercury for 2.5 years showed regeneration of the axons and myelinated nerve fibers (7). The neurons of the posterior ganglion are also usually well preserved in patients with Minamata disease (32–36) and in rats exposed to methylmercury (19). Therefore, it is reasonable to suspect that the peripheral nerves regenerate over the long term.

FIGURE 14b.—High magnification of a square part of Figure 14a. The figure shows a small axon with thick myelin sheath compared with the size of the axon in the regenerated nerve fiber. Bar = 1 \text{ \mu m}.

FIGURE 15a.—Longitudinal section of the sural nerve biopsy. Regenerated small axons encapsulated by the laminar processes of the Schwann cell cytoplasm are identified in the increasing collagenous fibers. Bar = 1 \text{ \mu m}.

It is important to consider the period of pollution of the methylmercury in Minamata Bay to understand sensory disturbances in Minamata disease patients. Sural nerve biopsies were performed in three Minamata disease patients in 1969, when only 1 year had passed since the end of the methylmercury discharge into Minamata Bay. Eto et al (1, 4) described incomplete regeneration of the sural nerves in these patients based on electron microscopy findings. In 1998, Nishimura (20) reported the major source of the methylmercury discharged from the Chisso Factory. The Chisso Factory had

FIGURE 15b.—High magnification of a square part of Figure 15a. The figure shows the thickly dense processes of Schwann’s cytoplasm in the regenerated nerve fiber. No myelinated lamella is found in the thickly dense processes of Schwann’s cytoplasm. Bar = 0.5 \text{ \mu m}.
used manganese dioxide as a cocatalyst from 1932 to 1951 in its acetaldehyde production plant. In 1951, however, the company changed the cocatalyst used in the process of producing acetaldehyde from manganese dioxide to ferric sulfide. This resulted in the production of methylmercury chloride, which the factory dumped directly into Minamata Bay from 1951 to 1968. Over those 17 years, Minamata Bay was contaminated with a high level of methylmercury, and the fish and shellfish in the bay accumulated methylmercury in their meat and internal organs (20, 36).

This present autopsy case is only one of 450 autopsy cases performed at the Kumamoto University School of Medicine, for which there are results of sural nerve biopsy and ultimate identification of typical lesions of Minamata disease at autopsy. Within 10 years, some patients of Minamata disease show complete regeneration of sural nerves. Nagaki et al. (16–18) reported the sural nerve biopsy findings of 8 patients with Minamata disease compared with 8 controls. These sural nerve biopsies of the patients were performed in 1981, over 20 years after the onset of Minamata disease. They found no statistical difference between the 2 groups in pathological and electrophysiological studies. Their conclusion was that Minamata disease patients had no peripheral nerve pathology. They hypothesized that the cause of the sensory disturbance might be damage to the sensory center of the post-central cortex. They also suggested that a lack of controls in an earlier study by 2 of the present authors (4) led to inappropriate interpretation of pathological changes of the sural nerves. It is reasonable to expect that regeneration of peripheral nerves over 20 years after the onset of Minamata disease would cause them to function within normal limits clinically and to be free of lesions. The findings of Nagaki et al. (16–18) probably reflect the complete regeneration of the peripheral nerves over 20 years after the onset of Minamata disease. Tokumoi et al. (38) reported similar results, in which typical Minamata disease patients surviving over 20 years showed disturbance of the sensory center rather than peripheral nerve injury. These results may in fact demonstrate repair of initial damage to peripheral nerves of Minamata disease patients.

REFERENCES


