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Encephalopathy in megacystis-microcolon-intestinal hypoperistalsis syndrome patients on long-term total parenteral nutrition possibly due to selenium deficiency

Received: 14 May 2001 / Revised: 22 April 2003 / Accepted: 22 April 2003 / Published online: 5 July 2003
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Abstract This report concerns two patients (female, 9 and 6 years) who were diagnosed with megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS). Although they exceeded the usual life expectancy of patients diagnosed with MMIHS because of total parenteral nutrition (TPN), they demonstrated progressive neurological deficits and showed histopathological features in the brain. Both patients were diagnosed with MMIHS in the neonatal period and were fed by TPN. The first patient developed visual and gait disturbances at the age of 7 years. Two months later, she developed dysarthria and muscular weakness, and could not maintain her posture. The level of serum selenium was extremely low. The second patient developed flexion and spasticity of the extremities fol-

lowed by decorticate posture at the age of 3 years. Both patients died of sepsis. The brain weights of the two cases were 880 g and 715 g. In both cases, severe neuronal loss and gliosis were present in the medial convolutions of the occipital lobe, including the visual cortex. The postcentral gyrus and temporal transverse gyrus were also involved. In addition, extensive loss of Purkinje cells and granular neurons, and gliosis were observed in the cerebellum. We measured the selenium content of the brains and livers using the graphite furnace atomic absorption spectrometry method. Selenium was not detected in either brain, although the livers of both cases contained a low level of selenium. On immunohistochemical examination of the anti-oxidative enzymes, histiocyte-macrophage lineage cells in MMIHS cases, including microglia and Kupffer cells, showed only a weak reaction for glutathione peroxidase, of which selenium is an essential component. However, the cells in the control cases were strongly positive. In cases of MMIHS and methylmercury intoxication, the brain features similar lesions, in both their topographical and histopathological aspects. We considered that the brain lesions of the MMIHS patients mainly resulted from oxidative damage of the brain related to the low levels of glutathione peroxidase and other selenoproteins due to selenium deficiency.

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Keywords Megacystis-microcolon-intestinal hypoperistalsis syndrome · Total parenteral nutrition · Encephalopathy · Selenium deficiency · Glutathione peroxidase

Introduction

Megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS) is an autosomal recessive disease characterized by a large urinary bladder, hydronephrosis, microcolon and hypoperistalsis. Since patients cannot be fed orally, they usually die before 1 year of age [2]. MMIHS belongs to the Hirschsprung's disease-related disorders, but ganglion cells and nerve fibers in the myenteric and submu-

cosal plexuses are often normal in number and have a morphology unlike Hirschsprung's disease [23]. Some authors have found degenerative changes of the smooth muscle cells in the bowel and bladder and have suggested that smooth muscle myopathy may be the cause [6, 11]. However, the pathogenesis of hypoperistalsis remains unknown. In the present report, we describe two cases of MMIHS, who lived to 9 and 6 years of age, sustained by total parenteral nutrition (TPN), but finally demonstrated progressive neurological deficits and showed distinctive histopathological changes in the central nervous system. Although a case of generalized axonal dystrophy has been reported to present MMIHS [1], the current cases demonstrated different histopathological features from those of the previous case.

Selenium deficiency state has been pointed out in patients fed on long-term TPN [17], and was thought to be related to progressive encephalopathy in a Crohn's disease patient on long-term TPN [15]. One of the present cases showed an extremely low level of serum selenium, and the clinical course and symptoms of the case resembled those of the patient with selenium deficiency. However, the histopathological features of selenium deficiency encephalopathy have not been described.

Selenium is an essential component of glutathione peroxidase (GPX), and is an integral part of the cellular equipment comprising catalase, superoxide dismutases (SODs), and α -tocopherol that prevent oxidative damage to tissues. Selenium deficiency results in a lowering of GPX, and this leads to characteristic cell damage [17].

In this report we present the histopathological features of the brain lesions in two MMIHS patients on long-term TPN. The brains appeared to share lesions with that of methylmercury intoxication, in both topographical and histopathological aspects.

To find out whether selenium deficiency was present, we measured the content of selenium in brain and liver. Furthermore, we investigated the expression of GPX with immunohistochemistry, and sought to clarify the pathogenesis of the distinctive brain lesions.

Case reports

Case 1

This patient was born in February 1984 after 37 weeks of gestation. Her birth weight was 3,510 g and it was a normal delivery. After delivery, a marked abdominal distention and vomiting were noted. She had not passed stools or urinated. An abdominal ultrasonography revealed bilateral hydronephrosis and marked distension of the urinary cyst. A gastrographin enema showed microcolon. She was diagnosed as suffering from MMIHS. A cystostomy was performed and the patient was fed by TPN. When she was 6 years old, she was discharged from the hospital, and went to elementary school for 4 months (being fed by venous nutrition at home). Her psychomotor development was not retarded. In August 1991, she developed visual and gait disturbances. Although an ophthalmological examination showed no abnormality, magnetic resonance imaging demonstrated focal atrophic lesions at the bilateral occipital lobes. The neurological abnormalities rapidly worsened, and in October 1991, she showed dysarthria, muscle weakness, spasticity of the extremities, and could not hold her posture. Her mental and

motor functions deteriorated. Laboratory examinations showed macrocytosis (mean corpuscular volume, MCV, 114 fl) with normal hemoglobin (14.2 g/100 ml). Vitamins and nicotinic acid were supplemented. Retinol-binding protein, prealbumin, transferrin and trace elements including manganese, copper and zinc were within the normal ranges. However, the level of serum selenium was extremely low (0.0–0.8 μ g/100 ml; normal 9.7–16.0). Since there were no signs of myocardopathy, selenium deficiency syndrome was not considered at that time. Occasionally she had convulsions and was treated with an anticonvulsant. She died in April 1993 of sepsis.

Case 2

This patient was born in August 1983 after 37 weeks of gestation. She was not related to patient 1. Her birth weight was 3,250 g and it was a normal delivery. At birth she had abdominal distention and became cyanotic. Ultrasonography revealed an intra-abdominal cyst and hydronephrosis. A gastrographin enema showed microcolon. As a result, she was diagnosed with MMIHS, a cystostomy was performed, and she was fed by TPN. In March 1984, she presented an ileus, and received surgical treatment. At that time, artificial ventilation was performed. At 15 months of age she had gained weight well. Although she was retarded, she showed significant psychomotor development such as standing, listening to stories, and looking at books. At 3 years there were some behavioral problems (i.e., a bad temper). Flexion and spasticity occurred, and the patient presented a decorticate contracture-like posture, following muscular atrophy. Brain computed tomography revealed brain atrophy, which gradually became more severe. Contracture worsened and body movement disappeared, and she cried without speaking. Management of her nutrition was difficult. Laboratory examinations revealed macrocytosis (MCV 130 fl) with normal hemoglobin (12.3 g/100 ml). Trace elements including manganese, copper and zinc were within the normal ranges. However, the level of serum selenium was not examined. She died of sepsis in August 1990.

Material and methods

Brain tissue was fixed in 10% phosphate-buffered formalin and embedded in paraffin wax. Paraffin sections were stained with hematoxylin-eosin, Klüver-Barrera, Bodian, Bielschowsky and Holzer methods. Brain lesions were immunohistochemically investigated using anti-glial fibrillary acidic protein (GFAP, polyclonal, our laboratory; 1:1,000), anti-S-100 protein (polyclonal, our laboratory; 1:1,000), anti-neurofilament 160 kDa (polyclonal, our laboratory; 1:500), anti-synaptophysin (monoclonal, Dako, Glostrup, Denmark; 1:10), LN-1: CDw 75 (monoclonal, Nichirei, Tokyo, Japan; 2:1), LN-3: HLA-DR (monoclonal, Nichirei; 2:1), Ki-M1p: CD68 (monoclonal, Seikagaku, 1:100), anti-cellular GPX (polyclonal, 1:1,000) [4], anti-Cu/Zn superoxide dismutase (polyclonal, 1:20,000) [3], and anti-Mn SOD (polyclonal, 1:10,000) [3] antibodies. The peroxidase-antiperoxidase method (Dako) and the biotin-streptavidin immunoperoxidase method (Nichirei) were performed for polyclonal and monoclonal antibodies, respectively. Prior to incubation with LN-1 antibody, sections were treated with 0.05% pronase in 0.05 M TRIS-HCl, pH 7.6 for 5 min. To compare the expression of GPX and SODs between the present cases and other brain lesions, brain and liver tissues obtained from three autopsy cases were also examined. These control cases included an 8-year-old patient with Schönlein-Henoch's purpura, a 5-year old patient with rhabdomyosarcoma, and a 5-year-old patient with neuroblastoma, show-

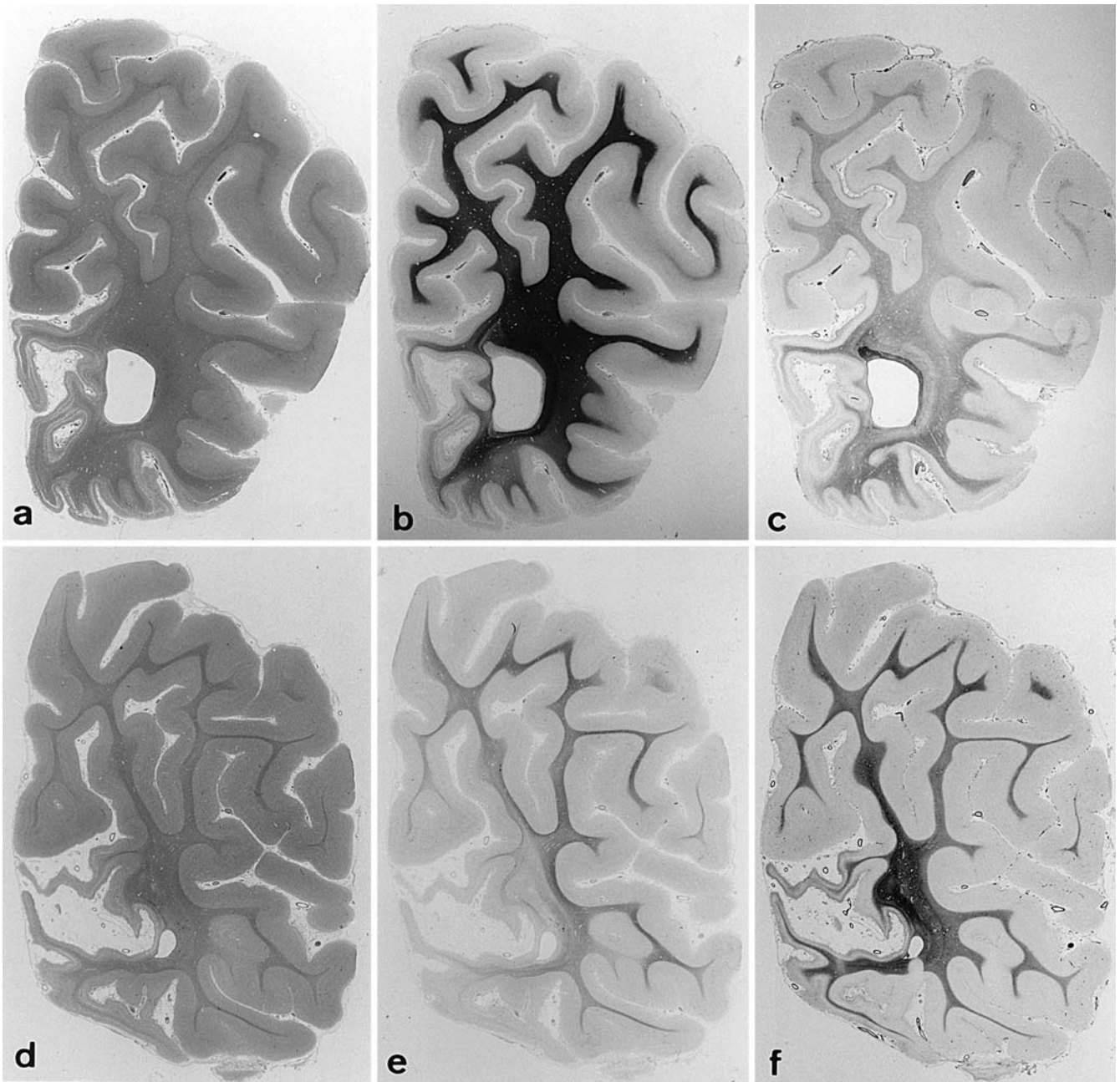


Fig. 1 a–c, d–f Coronal section of the left cerebral hemisphere at the level of occipital lobe in cases 1 and 2, respectively. Both cases show severe atrophy of the visual cortex, and the convolutions are severely attenuated in the second case especially. Moreover, the white matter is reduced in volume, showing myelin pallor and gliosis in case 2. **a, d** HE stain, **b, e** Klüver-Barrera (KB) stain, **c, f** Holzer stain; **a–c** $\times 1.3$, **d–f** $\times 1.4$

ing ischemic encephalopathy, mild subarachnoid hemorrhage, and mild meningitis, respectively.

Determination of selenium level in the brain and liver

Occipital lobe tissue and liver tissue, fixed in buffered formalin, from both cases were examined. Specimens from

the autopsy of a 6-year-old patient with Krabbe's disease were also examined. Selenium levels were determined using the graphite furnace atomic absorption spectrometry method. The method has been reported in detail previously [9].

Pathological findings

Case 1

Brain weight (unfixed) was 880 g. A macroscopic examination showed brain atrophy, especially in the bilateral medial occipital lobe including the visual cortex (Fig. 1a–c). No abnormalities were observed in the arteries forming Willis' ring and basilar arteries. In the attenuated gyri of

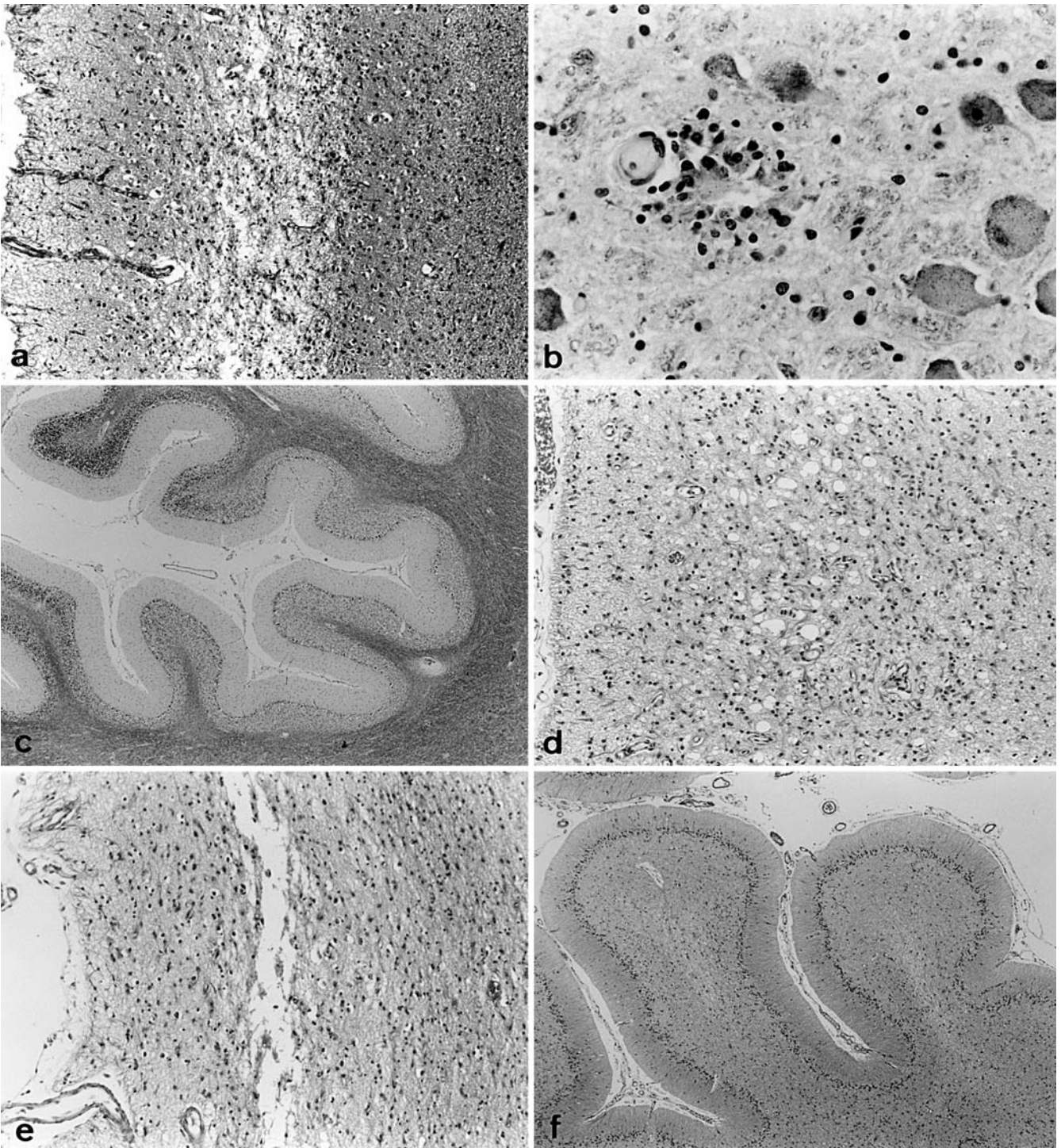


Fig. 2 a–c Histopathological features in case 1. **a** The cortex of the occipital lobe shows severe neuronal loss and astrocytosis, and the cortical parenchyma is loose in the middle layer. **b** Neuronophagia and central chromatolysis are seen in the locus ceruleus. **c** Cerebellar vermis exhibits a marked loss of granular cells, which is more advanced in the depths of sulci. **d–f** Histopathological features in case 2. **d** The cortex of the occipital lobe presents severe neuronal loss and astrocytosis in the whole layers, and spongy changes are present in the middle layers. **e** The cortex of the postcentral gyrus also exhibits severe neuronal loss and astrocytosis, and the neuropil is loose in the middle layers. **f** The cerebellar hemisphere shows severe loss of granular cells and Purkinje cells, and astrocytosis. **a, d–f** HE stain, **b, c** KB stain; **a, d, e** $\times 90$; **b** $\times 350$; **c** $\times 18$; **f** $\times 35$

the occipital lobe, extensive neuronal loss of the third to fifth layers and astrocytosis were observed, and the cortical parenchyma was loose in the middle layer (Fig. 2a). In the postcentral gyrus and temporal transverse gyrus, mild neuronal loss and astrocytosis of the third and fourth layers were present. There were no significant changes in the geniculate body and optic nerve. Neuronophagia and microglial nodules were present in several nuclei, including the thalamus, Meynert's nucleus, red nucleus and cuneiform nucleus. Microglia, surrounding degenerated neuronal cells (forming nodules), were immunostained with LN-1 and

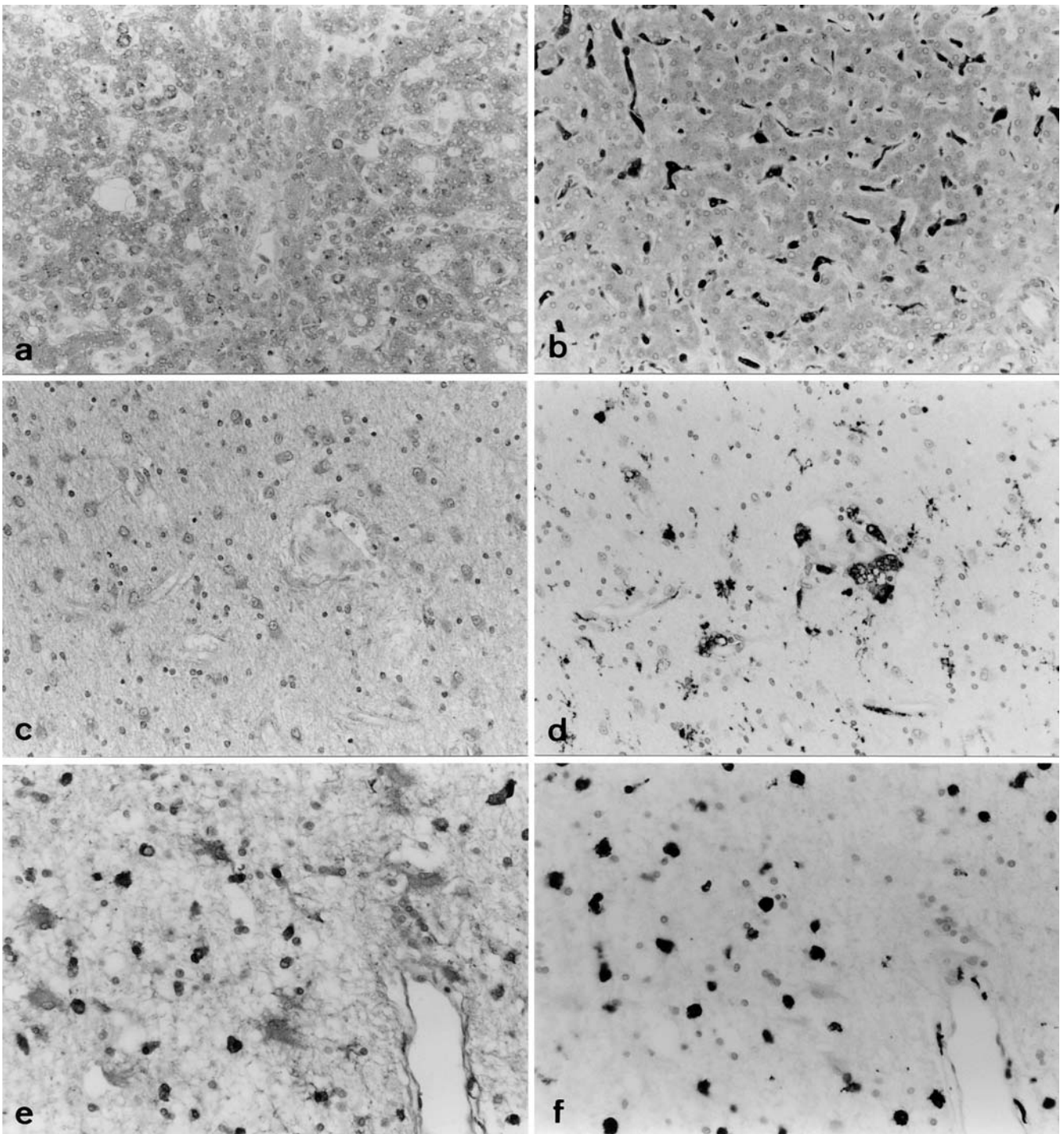


Fig. 3 **a** In the liver of case 2, hepatocytes and Kupffer cells are faintly positive for GPX. **b** In contrast, Kupffer cells in the control case are strongly positive for GPX. **c** The white matter of the occipital lobe in case 2. Reactive astrocytes are weakly positive for GPX. However, macrophages and microglia did not express GPX in this MMIHS brain. **d** On the adjacent section immunostained with KiM1p, a marker of microglia/macrophage, there are positively stained microglia and macrophages. **e, f** The white matter of the cerebrum in the control case developing ischemic encephalopathy. Compared with the adjacent section with KiM1p staining (**f**), macrophages and microglia are strongly positive, and reactive astrocytes are moderately positive for GPX (**e**) (GPX glutathione peroxidase). **a-f** $\times 180$

Ki-M1p. Central chromatolysis and neuronophagia were observed in the locus ceruleus (Fig. 2b). In the cerebellar vermis there was a severe loss of Purkinje cells and granular cells and a proliferation of Bergmann's glia (Fig. 2c). Torpedoes were observed in the cerebellar hemispheres. The dentate nucleus showed moderate neuronal loss and gliosis. The corticospinal tract developed pallor and decreased in volume at the levels of pons and medulla oblongata.

Case 2

The brain weighed 715 g, and was diffusely atrophic. In particular, the convolutions in the medial inferior part of bilateral occipital lobes, including the visual cortex, were severely atrophied (Fig. 1d–f). The postcentral gyrus was also attenuated. The white matter of the central semiovale was reduced in volume and the corpus callosum was thin. The lateral ventricles were enlarged. Vascular abnormalities were not observed. A histopathological examination revealed diffuse neuronal loss and gliosis in the atrophic occipital lobe and postcentral gyrus. In addition, a spongy state was observed in the middle layers of the severely affected cortex (Fig. 2d). The temporal transverse gyrus and the adjacent areas of the superior temporal gyrus showed a moderate loss and astrocytosis, and, in a part of the former, the lesions were observed in all the layers. The subcortical white matter showed a decrease in myelin and gliosis (Fig. 1d–f). Loss of nerve cells and gliosis were seen in the CA1, CA3 and CA4 areas of the right hippocampus. The lateral geniculate body showed a moderate loss of neurons and gliosis; however, the optic tract was not involved. The vermis and the dorsal part of cerebellar hemispheres showed an extensive loss of Purkinje and granular cells, and a proliferation of Bergmann's glia (Fig. 2f). Torpedoes were occasionally present. The dentate nucleus and the inferior olivary nuclei demonstrated a moderate loss of nerve cells and gliosis. A few microglial nodules were observed in the Meynert's nucleus.

Histological findings of the intestine

In both cases, the number and morphology of ganglion cells in the submucosal and myenteric plexuses were almost normal. The myenteric plexuses showed hyperplasia of nerve fibers at the rectum level, and they consisted of ganglion cells and peripheral nerve fibers. Histochemical examination of rectal biopsy specimens of both patients (taken while they were still alive) for acetylcholine esterase did not show positive fibers proliferating at the mucosal propria or around the mucosal muscular layer, unlike those of Hirschsprung's disease. No electron-microscopic examination of the smooth muscle cells in the muscular layer was performed.

Determination of selenium in brain and liver

The levels of selenium in the tissue specimens were measured in duplicate (by H.K. and A.Y.). The mean selenium concentrations in the livers of these MMIHS cases were 23.5 and 21.5 ng/g, respectively. However, in neither case was selenium detected in the cortex or the white matter of the occipital lobes. The cortex, the white matter of the occipital lobe and the liver of the Krabbe's disease patient demonstrated 99.3, 108.2 and 171 ng/g, respectively.

Distribution of GPX and SODs in brain and liver

In the livers of the MMIHS cases, GPX was faintly positive in both the Kupffer cells and hepatocytes (Fig. 3a). However, in the livers of the three control cases, Kupffer cells were strongly positive for GPX and the hepatocytes showed faintly granular staining (Fig. 3b). Staining for Mn SOD and Cu/Zn SOD revealed no conspicuous differences between the MMIHS and control cases. Mn SOD immunostaining demonstrated fine-granular positive products in the hepatocytes and Kupffer cells. The hepatocytes were diffusely immunoreactive for Cu/Zn SOD in both the nucleus and cytoplasm.

In the severe lesions of the MMIHS brains, reactive astrocytes were weakly positive for GPX, and the microglia and macrophages were unstained or only faintly stained (Fig. 3c, d). In contrast, in the normal cerebrum and brain stem of control cases, microglia, astrocytes and neurons were frequently positive for GPX. Microglia were more strongly positive for GPX than the other cell types. In the ischemic lesions of the cerebrum, microglia and macrophages showed a stronger reactivity with the anti-GPX antibody than reactive astrocytes (Fig. 3e, f). There were no significant differences between the MMIHS and control cases with regard to the expression of Mn SOD and Cu/Zn SOD. The SODs existed in the astrocytes, microglia and neurons.

Discussion

Both of the present cases exhibited distinctive lesions in the central nervous system. Severe neuronal loss and gliosis were present in the medial site of the occipital lobe, particularly in the visual cortex. The postcentral gyrus and temporal transverse gyrus were also involved. In addition, extensive loss of Purkinje cells and granular neurons and gliosis were observed in the cerebellum. Moreover, case 1 showed neuronophagia and microglial nodules in the thalamus, Meynert's nucleus, red nucleus, cuneiform nucleus and locus ceruleus. In case 2, neuronophagia was not as frequent as in case 1. In addition to these lesions, the CA1, CA3 and CA4 regions of right hippocampus were also involved. These features suggested that the etiology of the brain lesions was possibly attributable to hypoxia, a degenerative process specific to MMHIS or nutritional deficiency. Hypoxia was absent in the patient 1 and her psychomotor function developed sufficiently to attend an elementary school. In patient 2, artificial ventilation was performed during ileus surgery, and her psychomotor development was retarded. As neuronal loss was observed in the CA1, CA3 and CA4 regions, hypoxia may be partly responsible for the brain lesions in case 2. However, the cerebral lesions common to both cases were different from hypoxic encephalopathy in the aspect of the topography. To date the degenerative process in the central nervous system related to MMIHS has only been reported in one study [1], in which a generalized axonal dystrophy was described to present MMIHS, but the present cases

did not demonstrate the histological features of axonal dystrophy. Most of the authors suggested that hypoperistalsis was attributed to smooth muscle myopathy. Upon ultrastructural examination, some of them observed degenerative changes of the smooth muscle cells including excessive glycogen storage, vacuolar changes and reduced contractile myofibrils [6, 11]. However, Xu et al. [26, 27] experimentally demonstrated that mice with null mutations for the $\alpha 3$ neuronal nicotinic acetylcholine receptor and for $\beta 2$ and $\beta 4$ subunits of nicotinic acetylcholine receptors expressed a phenotype resembling MMIHS. Recently, Richardson et al. [20] confirmed a lack of $\alpha 3$ neuronal nicotinic acetylcholine receptor in tissue specimens from MMIHS patients. These receptors are more abundantly expressed in the peripheral autonomic neurons than in the brain. Based on those data, the main impairment in MMIHS may occur in the peripheral autonomic nerve or smooth muscle cells. It is questionable that the causes of impairment in smooth muscles or peripheral autonomic nerve generate progressive neuronal cell death (primarily in the sensory cortex and cerebellum).

The MMIHS patients 1 and 2 were fed by long-term TPN from birth until their deaths. The pathogenesis of the distinctive brain lesions may possibly be related to nutritional deficiency due to long-term TPN. However, vitamins and nicotinic acid were supplemented, and the levels of trace elements including manganese, copper and zinc were within normal ranges. The distribution and histopathological features of the brain lesions reported here are not consistent with those of the brain lesions due to nutritional deficiency that have been reported.

Most intravenous fluids used in TPN contain a negligible amount of selenium. Therefore, the patients on long-term TPN have been reported to demonstrate selenium-responsive syndromes including fetal congestive cardiomyopathy, peripheral myopathy, white fingernail beds and macrocytosis [17]. In patient 1, the blood selenium level was remarkably low, and both cases showed macrocytosis. Recently, Kawakubo et al. [15] have described irreversible progressive encephalopathy related to selenium deficiency in a Crohn's disease patient on long-term TPN. The patient initially showed visual disturbance, followed by various neurological symptoms (including paresthesia, dysarthria, and spasticity), and finally developed consciousness disturbance. A histopathological examination was not performed; however, the clinical course was similar to that of patient 1.

We measured the concentrations of selenium in the brain and the liver of the MMIHS patients and a patient with Krabbe's disease using the graphite furnace atomic absorption spectrometry method. The selenium concentrations of the cortex and white matter in the Krabbe's disease case were 99.3, 108.2 ng/g, respectively. These values are consistent with the reported values of the selenium concentration (99–274 ng/g) in human brain tissue [9]. However, in the brain tissues of the MMIHS cases selenium was not detected. The livers of the MMIHS patients showed much lower selenium concentration (23.5 and 21.5 ng/g) than that of the Krabbe's disease patient (171 ng/g). There-

fore, we considered that the brains of the current cases contained such low levels of selenium that they could not be detected. This indicates extreme selenium deficiency in the brains of the present cases.

The topography and histopathological features of the lesions in the present MMIHS cases resemble those of Minamata disease (methylmercury intoxication) [21, 24]. However, the present cases had no history of ingesting mercury, because they were on the long-term TPN without mercury. Several theories have been proposed concerning the pathogenesis of methylmercury neurotoxication. These include disturbances in the metabolism of protein and nucleic acids, disturbances on Ca^{2+} homeostasis, oxidative injury and aberrant protein phosphorylation [5]. Moreover, Skullerud et al. [22] have reported that a patient with generalized glutathione deficiency and pyroglutamic acid urea demonstrated this type, and distribution, of brain lesions after mercury intoxication. Glutathione is the substrate of GPX and glutathione S transferase, which prevents oxidative damage. They suggested that the damage was caused by the lack of protection by glutathione against oxidative damage. These facts suggested that the pathophysiological mechanisms of the brain lesions of the current cases were related to oxidative damage to the central nervous systems.

Selenium is an essential component of GPX (as well as several other selenoproteins), which is involved in antioxidant activity [7] and can alleviate the toxicity of methylmercury and favorably alter the biological and environmental fate of methylmercury [13, 19]. In an experimental study using rats fed on a selenium-deficient diet, selenium deficiency decreased the activity of lung GPX and exacerbated pulmonary injury following hyperoxic exposure compared with rats fed on a selenium-sufficient diet [14]. GPX is an integral part of the cellular equipment comprising catalase, SODs, and α -tocopherol, which protect tissues from oxidative damage by catalyzing the destruction of various toxic oxygen derivatives such as H_2O_2 , organic hydroperoxides, superoxide and hydroxyl radicals [17]. Thus, selenium deficiency results in a lowering of GPX activity. On immunohistochemical examination of anti-oxidative enzymes, there was a marked difference in the expression of cellular GPX between the present cases and the control cases. In the control cases, histiocyte-macrophage lineage cells including microglia and Kupffer cells were strongly positive for GPX; however, the MMIHS cases showed only a weakly positive reaction in the microglia and Kupffer cells. In the latter cases, microglia and macrophage were negative or faintly positive for GPX. Although reactive astrocytes were weakly positive for GPX, it was thought to be the nonspecific reaction. There were no differences between the control and MMIHS cases regarding the expression of Mn SOD and Cu/Zn SOD. It has been reported that GPX localized in microglia in some neurological disorders [7]. An investigation of excitotoxin-induced neurodegeneration also disclosed that cellular GPX immunoreactivity increased in activated microglia and in astrocytes in treated mice and that the globular-shaped microglia showed the most intensive immunoreactivity [16].

These findings were similar to those of ischemic encephalopathy in the control cases. Thus, the low expression of GPX in the histiocyte-macrophage lineage cells in the MMIHS cases may be related to a selenium deficiency. Lowering of GPX may lead to an increased susceptibility to oxidative damage. de Haan et al. have demonstrated that cellular GPX knockout mice were highly sensitive to the oxidant paraquat [8]. Toxic oxygen derivatives are also products of normal cellular activity, as well as products of the metabolism of foreign substances [18].

We consider that the encephalopathy of MMIHS patients may be attributed to selenium deficiency on long-term TPN without selenium supplement, and that the distinctive brain lesions may be formed due to oxidative damage.

The question of why the sensory cortexes, especially the visual cortex, are mainly involved in this pathological condition still remains unanswered. There are two possibilities; the neuronal cells in the areas may be more vulnerable to the oxidative damage, or they may have the metabolic tendency to generate more toxic oxygen derivatives than the other cortical neurons. The difference in neuronal vulnerability to oxidative damage among the areas of the cerebral cortex is unknown. Glucose is a main energy source to neurons in the brain, and oxidants are generated during normal metabolism including the oxidation of glucose [18]. We speculate that the difference in local rates of glucose metabolism may relate to the tendency to generate toxic oxygen derivatives. Humans receive a constant stream of sensory stimuli. While awake, this is especially true in regards to visual stimulation. Consequently, the sensory cortexes (especially the visual cortex) because of their constant use may have a glucose metabolic rate that is higher in total than that in other areas [10, 12]. It is speculated that intrinsic toxic oxygen derivatives may be generated more in the sensory cortexes, resulting in these areas being more impaired than the other areas. In an experimental study of rat brain, the primary sensory areas exhibited the highest local cerebral glucose utilization in layer IV [25]. The current cases showed severe neuronal loss, astrogliosis, and a spongy state mainly in layers III-IV of the sensory cortexes. Although investigation of the same aspect of the human brain has not been performed, the topographical coincidence supports the notion that a different rate of glucose metabolism may also be attributed to the laminar distribution of the cortical lesions of the present encephalopathy.

Acknowledgements The authors wish to thank Dr. Hitoshi Takahashi (Department of Pathology, Brain Research Institute, Niigata University), Dr. Noriyuki Shibata (Department of Pathology, Tokyo Women's Medical College), Dr. Hideaki Imai, and Dr. Masafumi Hirato (Department of Neurosurgery, Gunma University School of Medicine) for their significant suggestions and Mrs. Machiko Yokota and Mr. Koji Isoda for their excellent technical assistance. We also thank Mr. Roland Reeves for helping us to complete this manuscript.

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