Neuropathology of Mitochondrial Encephalomyopathies Due to Mitochondrial DNA Defects

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INTRODUCTION

Mitochondria are the major source of energy in all mammalian cells, and they contain a unique genome that is maternally inherited (1, 2). Mitochondrial DNA (mtDNA) is a 16,569-base pair, double-stranded, circular molecule that encodes 22 transfer RNAs, two ribosomal RNAs and 13 key subunits of the respiratory chain: seven subunits of Complex I, one subunit of Complex III, three subunits of Complex IV (cytochrome c oxidase) and two subunits of Complex V (ATP synthase). These subunits are synthesized within the mitochondron where they are assembled together with their nuclear counterparts, which are encoded by nuclear DNA (nDNA), synthesized in the cytoplasm and transported into the mitochondria (3).

Mitochondrial encephalomyopathies comprise diverse clinical disorders associated with structurally or functionally abnormal mitochondria in all tissues, but especially affecting muscle and brain, probably because of the high dependence of these tissues on oxidative metabolism (4). Among these disorders Kearns-Sayre syndrome (KSS), myoclonic epilepsy with ragged-red fibers (MERRF) and mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS) are the most distinctive syndromes (5–7).

Recently, specific mutations of mtDNA have been found for each syndrome. Deletions of muscle mtDNA have been documented in most patients with KSS (8, 9); a point mutation in the tRNA Lys gene, first described in members of one large pedigree with MERRF, has been confirmed in many other patients (10, 11); and Goto et al (12) and Kobayashi et al (13) have described a point mutation in the tRNA Leu (UUR) gene in muscle from several patients with MELAS.

Morphologically, ragged-red fibers (RRF) in muscle are associated with various neuropathologic alterations in the brain. These lesions tend to be localized in different regions of the central nervous system (CNS), thus explaining, in part, the different clinical manifestations that characterize the three syndromes.

The purpose of this review is to describe the neuropathologic alterations of the CNS in KSS, MERRF and MELAS using data obtained from our own material and published autopsy cases (14–58). The article is not intended to be comprehensive, since recent reviews have covered the clinical and molecular genetic aspects of the syndromes in detail (59–61).

HISTOPATHOLOGY

The general histopathology of mitochondrial encephalomyopathies consists of nonspecific lesions which form distinctive pathologic entities by their expression in different topographic patterns (Table 1). KSS is a degenerative multisystem disorder characterized by spongiform encephalopathy, neuronal degeneration, astrocitosis and, less frequently, demyelination. MERRF is a “system degeneration” (44, 62) with more selective neuronal degeneration, astrocitosis and degeneration of myelinated tracts. Multifocal necrosis, mineral deposits, neuronal degeneration and status spongiosus are typical of MELAS.

Status Spongiosus

This term is used to describe vacuolation of nervous tissue, predominantly of white matter, resulting in a sieve-like appearance (63) (Fig. 1). By light microscopy, the vacuoles generally appear ovoid with major axes parallel to axon fibers and measure from 30 to 260 microns in diameter. No material stainable by Hematoxylin-Eosin, Alcian Blue, Luxol Fast Blue-PAS, Sudan-Black B or Toluidine Blue is present in the vacuoles (17, 27, 29, 30, 32). Oligodendrocytes are usually preserved and myelin breakdown products are lacking or sparse.

In KSS, status spongiosus has been the most typical lesion. It was present in virtually all cases, except for some biopsies (20, 26). Both white and gray matter of several anatomic sites in the CNS were affected to varying degrees. The white matter was affected almost exclusively in the cerebrum and cerebellum, while involvement of gray matter was preferred in the brain stem. In the spinal cord, the lesion usually involved both white and gray
TABLE 1
Topographic Distribution of Brain Lesions in KSS, MERRF, and MELAS

C.Co. = cerebral cortex; C.WM = cerebral white matter; B.G. = basal ganglia; DIEN. = diencephalon; Cb.Co. = cerebellar cortex;
D.N. = dentate nucleus; Cb.WM = cerebellar white matter; M.B. = midbrain; PO. = pons; MED. = medulla; S.GM = spinal cord gray matter; S.WM = spinal cord white matter.

KSS

MERRF

MELAS

- STATUS SPONGIOSUS
- NEURONAL DEGEN.
- GLIOSIS
- DEMYELINATION
- NECROSIS
- MINERAL DEPOSITS
matter to the same extent. Topographically, the spongiiform lesion affected, in order of frequency, brain stem tegmentum, white matter of cerebellum and cerebrum, cervical spinal cord, basal ganglia and diencephalon (Table 1). In the cerebrum, the white matter was diffusely affected, except for one case in which there was a patchy distribution (23). The involvement of U fibers and subcortical white matter was reported in six cases (18, 24, 28–30, 37), and Goodhue et al (30) and Bresolin et al (35) described spongy changes in the deeper cortical layers of the cerebrum.

In MERRF, status spongiosus was occasionally present in the cerebral hemispheres (37–39) and was described once in the subcortical white matter of the cerebellum (39).

In MELAS, spongy degeneration involved preferentially the cerebral cortex (46, 48, 52, 53) and the lateral and posterior columns of the spinal cord (50, 52). In Ihara’s report (55) sponginess was noted in the cerebral white matter, optic nerves and pons.

Neuronal Degeneration

Neuronal involvement in KSS, MERRF and MELAS ranged from generalized atrophy to the more frequent
neuronal loss (Fig. 2). Ballooning of the perikarya and central chromatolysis were described in a few cases of MELAS (47, 48, 52) and in one patient with MERRF (38).

In KSS, neuronal degeneration has been the second lesion in order of frequency and affected preferentially the brain stem and the cerebellum (Table 1). Involvement of thalamus and basal ganglia has been noted in a few cases (16–18, 22, 29, 36, 37); only two authors (16, 17) reported decreased numbers of neurons in cerebral cortex. In the brain stem, the most affected regions were the substantia nigra, the red nucleus, the vestibular nucleus and the oculomotor nucleus. In the cerebellum, loss of Purkinje cells was common (Fig. 1), while numerical de-
crease of cells in the dentate nucleus and atrophy of the molecular layer were rare (17, 19, 27, 30, 36).

The topography of neuronal degeneration in MERRF also showed preferential involvement of cerebellum, brain stem and spinal cord (Table 1). In the cerebellum, neuronal loss was constant in the dentate nucleus (Fig. 2A), milder and variable in Purkinje cells (39–42, 44) (Fig. 2B), and rare in the granular layer (38, 42). The inferior olivary nucleus was the most severely affected in the brain stem, followed by the red nucleus and substantia nigra. Neuronal loss was also frequent in the gracile and cuneate nuclei (37–39, 41, 42), while it was less common in the vestibular nucleus and locus coeruleus (37, 39, 40, 42, 43). In the spinal cord, there was significant reduction of cells in Clarke’s nucleus and mild loss in the anterior and posterior horns (38–42). Mild involvement of basal ganglia and diencephalon was noted in three cases (38, 41, 42). Only Fukuhara (41) described a significant loss of neurons in the cerebral cortex.

In MELAS, neuronal degeneration also occurred independently of the necrotizing process described before. The cerebellum was the most affected structure with neuronal loss in the cortex (46, 48, 50, 52–54) and dentate nucleus (48, 55). In cerebral cortex, neuronal loss was reported twice (46, 48), and nonspecific atrophy was described by Hasuo et al (51).

Focal Necrosis

Although necrotic lesions in various sites have been found in four cases of MERRF (37, 38, 44) and two of KSS (22, 24), this lesion has been considered characteristic of MELAS. In this disease, focal necrotic lesions were always present and characterized by multiple softenings, solitary or confluent, varying in size and age (Fig. 3A, B).

Focal necrosis was missing only in the case of Hart et al (46) and Hamazaki et al (54), who described lesions ranging from microcystic rarefaction to extensive laminar necrosis of the cerebral cortex. Histologically, these lesions displayed loss of neurons, microreactive astrocytosis or cavitation and appeared to represent infarctions (45, 47, 52, 55, 56, 58). In affected areas, capillary prominence or overt proliferation was also frequent (45, 47, 51, 55, 56), while spongiiform was noted only in one case (52).

Topographically, bilateral involvement of cerebral cortex (Table 1) was constant and softenings were usually visible at the top of the cerebral gyri, but not in the depths of the sulci (55). Necrotic foci were also present in subcortical white matter (47, 52, 55), basal ganglia (48, 50, 52), thalamus (48, 52, 58), cerebellum (52, 53, 58) and brain stem (52, 55). There was, however, no evidence of thromboembolism nor good correlation between distribution of the lesions and major vascular territories (47, 52).

Astrocytosis

In KSS, MERRF and MELAS astroglialosis was also frequent and with a pattern of distribution generally related to the previously described lesions (Table 1). Occasionally, swollen and misshapen astrocytes (16) or Alzheimer type II cells (18, 24, 29, 30) were found in the most affected areas.

Demyelination

The presence and intensity of demyelination in KSS was generally related to the severity of spongy degeneration (Table 1). The most affected areas included white matter of cerebrum and cerebellum and posterior columns of spinal cord.

In MERRF, tract degeneration with consequent loss of myelin preferentially affected the superior cerebellar peduncles and posterior columns. In spinal cord, degeneration of the posterior spinocerebellar tracts was also common and severe (38, 40–42); pyramidal involvement, when present (38, 40, 42), was mild.

In MELAS, demyelination was seldom present in the cerebral white matter (53, 54) and in spinal cord (48, 49, 58). Mizukami et al (58) reported focal demyelination with spheroids in the pontocerebellar fibers of the pons, but typical central pontine myelinolysis was described only in one case (53).

Mineral Deposits

Pallidal and nigral siderosis was first described in KSS by Kearns and Sayre (14). Mineral deposits in globus pallidus and thalamus have been described in seven other cases of KSS (17–19, 30, 32, 36, 37), but not in any cases of MERRF. They consisted of finely granular siderotic deposits usually localized in and around capillary and arterial walls.

In MELAS this has been the second most frequent lesion. Mineral deposits were common in the basal ganglia, especially in globus pallidus (Fig. 3B, C) (45–48, 52–55), and less frequent in thalamus (47, 52), dentate nucleus (47, 48, 52) and midbrain (47, 52).

Less Frequent Histopathologic Lesions

Vascular Proliferation: In KSS, vascular proliferation was described in four cases with a variable topographic pattern: cerebral white matter, basal ganglia, thalamus, substantia nigra and medulla (17, 23, 33, 36).

In MERRF this lesion was found in the necrotic lesions of Fukuhara’s first report (38) and in the third case of Berkovic (44). In the latter, the lesions were present bilaterally in brain stem, characteristic of Leigh’s syndrome. They showed marked rarefaction of the neuropil, relative neuronal preservation, astrocytosis and excessive thin-walled vessels.

Capillary proliferation in MELAS was frequent in and
around necrotic foci (45, 47, 51, 55). In one case, it was prominent in the dentate nucleus (48).

Axonal Spheroids: Axonal spheroids have been found in the cerebellar granular layer and white matter, substantia nigra, globus pallidus, and gracile and cuneate nuclei in some patients with KSS (17, 18, 24, 30, 37) and MERRF (37, 42).

Gumose Degeneration: This lesion (64) has been detected rarely in the cerebellum of MERRF cases (39, 41).

ULTRASTRUCTURAL PATHOLOGY

Ultrastructural studies of the CNS have been performed only in a few cases.

In KSS such studies generally failed to show any mitochondrial abnormalities (20, 26, 28, 31, 35-37). In one case, there were enlarged mitochondria with abnormal cristae and paracrystalline inclusions in Purkinje cells (20). The spongy vacuolation in hemispheric white matter was intramyelinic with splitting of the myelin sheaths at the minor dense line (36, 37).

An ultrastructural study of an autopsied case of MERRF (43) also failed to reveal abnormal mitochondria in the CNS. However, Fukuvara (62) described enlarged mitochondria with vesicular inclusions in the cerebellum and dentate nucleus of a 30 year old woman who died of MERRF; a complete pathological report of this case has not been published.

In MELAS, increased numbers of structurally abnor-
ormal mitochondria were found in endothelial and smooth muscle cells of blood vessels (52, 53, 55, 58). These changes appeared to be most prominent in the walls of pial arteries and small arteries up to 250 microns in diameter (52). There is only a single report of abnormal mitochondria in the neurons of cerebral cortex, Purkinje cells, internal granular layer cells, astrocytes and oligodendrocytes (55).

CELLULAR PATHOPHYSIOLOGY

Mitochondrial diseases caused by mtDNA mutations are characterized by the presence of a mixture of wild type and mutant mtDNAs in cells (heteroplasmy) (60). At the time of cellular division, the proportions of the two types of mtDNAs, and thus the mitochondrial genotype, can shift because of the random partitioning of mtDNAs into daughter cells (replicative segregation) (59, 60). The phenotypic expression of a mtDNA mutation is dictated by the "threshold effect," that is, the mutant phenotype (impairment of oxidative phosphorylation) is expressed in the heteroplasmic cells only when the relative proportion of mutant mtDNAs reaches a certain value (59, 60, 65). This phenomenon seems to regulate the expression of the pathology at the cellular level in mitochondrial diseases. It remains to be elucidated, however, how the impairment of oxidative phosphorylation leads to irreversible cell injury.

As described in anoxic conditions, the failure of energy-dependent ionic pumps could disrupt normal ion homeostasis with potassium efflux and sodium, chloride and calcium influx (66). A rise in intracellular free calcium concentration is associated with phospholipase activation, breakdown of cell membranes, and release of free fatty acids as arachidonic acid (67). The metabolism of arachidonic acid by oxidases leads to the production of free radicals and further cellular damage (67, 68).

The "weak excitotoxic hypothesis" of Albin and Greenamyre (69) could also explain the pathogenesis of neuronal death in mitochondrial encephalomyopathies. Henneberry et al (70) have shown that neurons in culture are normally resistant to the excitotoxic effects of glutamate. However, if these neurons are deprived of glucose, they become vulnerable to the excitotoxic effects of glutamate acting via N-methyl-D-aspartate (NMDA) receptors. Impaired translation of mtDNA-encoded subunits of the respiratory chain leading to defective energy production has been documented in cell lines derived from patients with KSS, MERRF and MELAS. Presumably the impairment of oxidative phosphorylation leads to failure of the Na/K ATPase with subsequent membrane depolarization, reduced magnesium blockage and activation of NMDA receptors.

If the "weak excitotoxic hypothesis" applies to the mitochondrial encephalomyopathies, the process of neuronal death could be initiated when the relative proportion of mutant and wild type mtDNAs reaches the threshold necessary to impair oxidative phosphorylation, and excitotoxicity would be the final common pathway of neuronal death. Inter- and intraregional differences in neuropathology in KSS, MERRF and MELAS could be explained if specific populations of neurons or glial cells were more severely affected in each of the three syndromes. Defective oxidative phosphorylation in specific populations of neurons would, in turn, render them susceptible to the excitotoxic effects of excitatory amino acids (EEA). Abnormalities of energy metabolism in supporting cells (astrocytes and oligodendrocytes) and in cells of the blood vessels could enhance vulnerability to NMDA receptor-mediated excitotoxic neuronal death.

The concept of excitotoxic neuronal death due to overstimulation of EAA receptors has become a fashionable pathogenetic hypothesis for neuronal death in a variety of neurological disorders (69, 71). Numerous lines of evidence support the role of excitotoxicity in acute processes such as hypoxia/ischemia and hypoglycemia (71, 72), but the role of excitotoxicity in chronic neurological diseases, including mitochondrial encephalomyopathies, is not firmly established.

However, the availability of drugs that block or inhibit NMDA receptor activation opens interesting therapeutic approaches. If excitotoxicity is the mechanism leading to neuronal death in mitochondrial encephalomyopathies, then it might be possible to use drugs that block the EAA receptors and thereby interfere with the mechanism of neuronal death in these ultimately fatal disorders.

TISSUE PATHOPHYSIOLOGY

At the tissue level, a failure of oxidative phosphorylation and the consequent lack of oxygen utilization seem to create a condition of "severe but incomplete ischemia" (73), that is, tissue anoxia in the presence of continued blood supply. The persistent supply of glucose to the tissue, permitting continued glycolysis, may cause a rise of local lactate levels and tissue damage (73, 74). According to this hypothesis, the degree and nature of the focal lesions could be related to the number of cells involved per unit of time. For instance, rapid damage of numerous cells could explain the infarctive nature of the brain lesions in MELAS. In this syndrome, the possible alteration of cerebral blood flow autoregulation (52, 75) could represent a further aggravating factor.

Status spongiosus is the most frequent CNS lesion in mitochondrial encephalomyopathies. The involvement of different cell types may account for its differential expression in white and gray matter. Spongy degeneration of white matter seems to result from splitting of the myelin sheaths at the minor dense line (36, 37) and could be related to a metabolic alteration of oligodendrocytes (36). Similar lesions have been reproduced in animal models with the administration of drugs that impair mi-
tochondrial function, such as ouabain, ethidium bromide and cuprizone (76-78). In contrast, there are no ultrastructural studies of status spongiosus in gray matter of patients with mitochondrial diseases. We can hypothesize that astrocytic swelling may occur, as in the Canavan's disease, where abnormal mitochondria in astrocytes have been identified (63, 79, 80).

The precise mechanism leading to the different regional expression of neuropathologic lesions in mitochondrial encephalomyopathies represents a central problem to be solved. Several factors may play a role in its determination:

1) uneven distribution of wild type and mutant mtDNAs in the stem cells of different neuronal populations during ontogenetic development (81, 82),
2) different cellular thresholds to metabolic damage in the various areas of CNS according to the local dependence on oxidative metabolism, and
3) selective vulnerability of CNS cells to local toxic factors, such as lactate or EAAs.

CONCLUDING REMARKS

The neuropathologic characteristics of KSS, MERRF and MELAS appear related not to specific single lesions but rather to different topographic expressions of common lesions.

The rarity of the diseases and cellular postmortem artefacts have limited ultrastructural and biochemical studies and justify the paucity of relevant data in the literature.

The utilization of new morphologic methods, such as immunological and molecular genetic probes, will provide insight into the pathogenesis of cellular dysfunction and will contribute to a better definition of these diseases from the morphologic point of view.

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