Ultrasonic Alterations in the Spinal Cord of Brown Swiss Cattle Affected with Bovine Progressive Degenerative Myeloencephalopathy (Weaver)

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Introduction

In the group of neurodegenerative diseases of cattle several inherited diseases have been described. The mechanisms underlying most are unknown. Some have been traced to metabolic causes like mannodisosis in purebred Angus,[15] GM, Gangliosidosis[13] and generalized glycogenosis.[22-26] Other diseases such as hereditary neuraxial edema, congenital brain edema, spastic syndrome, "shaker calf," inherited epilepsy, "doddlers" and progressive spinal myelinopathy,[25] have been classified upon the clinical, pathological and genetic criteria.[7]

Bovine progressive degenerative myeloencephalopathy (BPDME, "weaver") in purebred Brown Swiss cattle of the United States has been described in 1973.[18] Since then, similar cases have been reported from Denmark[14] and Switzerland.[5,24] A similar disease has been described in an Angler heifer.[13] The disease exhibits a familial pattern and has an onset at five to eight months of age. It affects both sexes and is characterized clinically by hind leg weakness, ataxia, and dysmetria in the absence of other significant abnormalities.[34] The animal's locomotor function deteriorates progressively and inability to rise follows and secondary fatal complications may develop.[32,33]

Microscopic studies have described axonal degeneration in the white matter of the spinal cord[5,19,34] particularly in the thoracic segments[34] and cerebellar lesions consisted in loss of Purkinje cells. Axonal swelling has been observed in the brain stem nuclei and medulla oblongata.[34] Recent study confirmed and marked axonal degradation in the spinal white matter which involved mainly the describing tracts.[5]

This report describes histopathological and ultrastructural findings in the spinal cord of Brown Swiss cattle affected with BPDME ("weaver syndrome").

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Materials and Methods

Experimental Procedures

Twelve Brown Swiss cattle, 7 to 30 months of age, ten with clinical manifestations of weaver syndrome and two normal controls were studied. All cattle were euthanatized by intravenous injection of T61 solution and the spinal cords removed immediately from each animal. Within 15 minutes of death, thin slices of the thoracic portion of the spinal cord were placed in cold glutaraldehyde in 0.05 M sodium cacodylate buffer (pH7.3). White and gray matter were separated and diced into 1 mm^3 pieces. Tissue samples were transferred to individual vials containing fresh, cold glutaraldehyde-cacodylate fixative, where they were held for one hour at 2°C. The initial fixative was replaced with cold, fresh glutaraldehyde-cacodylate in which tissues were fixed for one to two additional hours.

Preparation of Samples for Electron Microscopy

Fixed tissues were rinsed three times in sodium cacodylate and retrimmed, if necessary. Tissues were postfixed in buffered 1% osmium tetroxide, washed three times with distilled water, and dehydrated through a graded ethanolic series to propylene oxide. Tissue samples were infiltrated and embedded in Epon (LX112). Thick and thin sections of embedded tissues were cut, using an ultramicrotome (Sorvall MT2). Thick sections were stained with toluidine blue for light microscopy. Thin sections were stained with ethanolic uranyl acetate, followed

by triple lead stain.[1] Sections were examined with a Hitachi H300 transmissions electron microscope. Electronmicrographs were prepared according to standard darkroom procedures.

Results

Clinical findings

The disease was characterized by hind leg weakness, ataxia, and dysmetria in the absence of other significant clinical abnormalities. The animals lost control of movement and later the ability to stand.

Gross pathological lesions

Macroscopic pathologic changes were not significant.

Light microscopic changes

Examinations of semi-thin sections revealed no inflammatory or vascular changes. The most striking changes were consistently found in the ventral funiculi of the thoracic segments of the spinal cord.

Wallerian degeneration was present at all levels of the spinal cords; however was most severe at the thoracic level. Axonal swelling and myelin degradation conferred to the white matter a diffuse, spongy, vacuolar appearance. There was wide variation in the thickness of myelin sheaths. Degenerated axons contained axoplasmic remnants and were covered by sheaths that were thin and condensed or split, vacuolated, swollen and corrugated. In longitudinally sectioned fibers, fragments of compacted myelin fragments were present along the axis of the axon. Phagocytic cells engulfing myelin debris, and glial cells were observed in area of degeneration.

Electron microscopic changes

Axonal changes were consistently observed. The most severe alterations were found in the thoracic spinal segments. Axonoplasmic atrophy was observed frequently. The axoplasm was small and displaced to the periphery by edema in the periaxoplasmic space. Fragmented axoplasms containing abnormal structures were also observed.

Accumulation of abnormal axoplasmic structures

The axoplasm of degenerating axons frequently showed accumulation of neurofilaments, degenerated organelles, laminated bodies, vacuoles and dense inclusions.

Myelin sheaths

Variation in myelin thickness appeared to be a prominent change. Myelin changes were not necessarily associated with axoplasmic alterations. Degenerating myelin was vacuolated with separation of myelin lamellae. This change was present in either the axoplasmic side of the myelin sheath or concerned its whole thickness. Collapsed myelin sheaths were also observed.

Demyelinated axons

There were partially naked axons. Degenerated myelin was present in the periphery of the axoplasm. Myelin debris were taken into chambers in the axoplasm by phagocytic cells.

Cellular changes

Glial cells were observed in areas of degenerating axons.

Reactive astrocytes: These cells were characterized by astroglial filaments. Their cytoplasmic processes contained myelin debris vacuoles and glycogen granules.

Oligodendrocytes

These cells had intracytoplasmic, dense, osmophilic bodies.

Phagocytic cells

Degenerated myelin was engulfed by phagocytic cells. Some phagocytic cells were present in the axis cylinder. Cells with intracytoplasmic digestion chambers containing myelin debris, phagolysosomes and lipid droplets were observed.

Neurons

Changes observed in the neurons included dense laminated structures in a dendrite and "zebra-like" bodies in the perikaryon.

Discussion

Clinical findings

The age of onset, course and neurologic disturbance, were identical to those described previously.[18,19,32-34] A significant number of inherited neurodegenerative diseases of cattle may be present in this manner and includes diseases involving accumulation of storage products as in mannosidosis in purebred Angus, GM, gangliosidosis and neuronal lipodystrophy of Beefmasters.[7] These diseases are characterized by the accumulation of PAS positive material in saccular dilatation of the Golgi apparatus in neurons, astrocytes, pericytes of blood vessels and macrophages.[28] Other diseases are progressive ataxia in Charolais cattle—histologically characterized by formation of eosinophilic plaques in the white matter of the central nervous system.[9] Hereditary neuraxial edema[10] in neonatal polled Hereford is represented by microscopic spongy vacuolar appearance of the nervous tissue along the long axis of myelinated fibers in the white matter.

The "shaker" calf syndrome, spastic paresis, spastic syndrome, "doddlers" syndrome progressive spinal myelinopathy[25] have been described but are clinically and pathologically different from the weaver syndrome in the

Brown Swiss cattle.[7]

Pathologic findings

Light microscopy: histopathologic lesions in the spinal cords of Brown Swiss weavers were identical to those described previously.[34] The axonal swelling we have observed has been reported in a great variety of conditions in humans and animals, and is thought to reflect a nonspecific reaction of the neuron to a variety of noxious stimuli including chemical, genetic, and traumatic agents.[16] These factors may perturb the metabolism or function of the axoplasm. Under light microscopy these axonal swellings are hard to distinguish from other non-specific enlargements.

The cause of axonal atrophy is the impairment of the slow axonal transport responsible of the delivery of neurofilaments.[8] The axoplasmic alteration observed in the longitudinally sectioned fibers may represent a segmental degenerative process. This process is known to occur in toxic neuropathies such as lead neuropathy and diabetic neuropathy.[23]

Abnormal intra-axonal bodies have been described in dystrophic axons and during many toxic neuropathies.[21] They appear to be common non-specific evidence of nerve degeneration.[30] The accumulated intra-axonal bodies are probably derived from degenerating organelles like mitochondria. This maybe responsible for impaired respiration secondarily causing interference with energy dependent axonal transport.[30]

Various axoplasmic abnormalities have been described in the inherited necrotizing myelopathy of Afghan hounds.[2] Degenerating mitochondria with accumulation of electron dense material have been observed in methyl mercury poisoning and hypoxia.[16] Accumulation of mitochondria may be observed during early stages of Wallerian degeneration.[36] Laminated structures similar to those we have described in a dendrite have been described in canine gangliosidosis.[27]

Phagocytosis

Two mechanisms, by which necrotic axoplasms are removed have been proposed. Endogenous lysosomal activity and heterophagocytosis.[3] Oligodendrocytes like schwann cell have been implicated in the sequestration and removal of debris in neuropathies.[4,21] Other known potential phagocytic cells are macrophages derived from blood monocytes,[29] astrocytes and "multipotential glial cell."[17,35] We have described macrophages and activated astrocytes containing degraded myelin fragments, lipid droplets and vacuoles.

Myelin sheath

Degeneration myelin we have observed was characterized by vacuolation, disorganization and collapse of its lamellar structure. Similar changes have been described in many toxic axonopathies. Intramyelimic vacuolation has been observed in laboratory animals experimentally intoxicated by a copper chelating agent—cuprizone, hexachlorophene and triethyltin.[31] In the latter the edematous change involves separation of lamellae along interperiod lines and the formation of large fluid filled intramyelimic splits.[23]

Pathogenic mechanisms underlying this defect are not known. It is hypothesized that a failure of transport system or disruption of energy producing mechanisms may operate in these induced pathologies.[11] In the case of cuprizone it is thought that the formation of abnormal DNA dimers leads to a defect in mitochondrial respiration.[20] Intramyelinic vacuolation has also been described in calves with experimentally induced hyperammonemia.[6]

In addition to toxic insults, conditions under which myelin degenerates include: mechanical factors, immune response, genetic defect that manifests itself prior to or after the formation of myelin or metabolic defect in the myelinating cell.[23]

Partially naked axons we have observed may represent a secondary demyelination. This change was not predominant. The consistent change was Wallerian degeneration and involved simultaneous loss of both myelin and axons. The defect in the central nervous system of affected animals produces Wallerian degeneration of spinal cord white matter. More evidence should be accumulated based on pathological and biochemical data in order to determine the pathogenic mechanisms underlying this degenerative process.

Summary

Histologic and ultrastructural features of bovine progressive degenerative myeloencephalopathy (BPDME) in Brown Swiss cattle are described. There was evidence of axonal degeneration with predominance of simultaneous degradation of both axon and myelin. Phagocytic cells were present in areas of degeneration and were active in removing axonal debris. These lesions are not specific to the BPDME and can be classified as Wallerian degeneration.

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