Histopathology of Congenital and Perinatal Cerebellar Anomalies in Twelve Calves

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ABSTRACT. Congenital and perinatal cerebellar anomalies of 12 calves were morphologically divided into four groups. Three calves showing minor histological changes such as heterotopia of the cortex or defect of the cortical layers localized in a cerebellar gyrus were diagnosed as Akabane disease. The other two cases were characterized by bilateral partial defects of cerebellar tissue in the lateral hemispheres. The remaining three and four cases showed hypoplasia and degeneration in the cerebellum, respectively. Cerebellar hypoplasia of the former three calves was considered to be caused by BVD-MD virus infection. A pathogenesis of the cerebellar degeneration seemed diverse resulting from hypoxia during a perinatal period or increased intracranial pressure due to dysplasia of the ventricular system.—KEY WORDS: Akabane disease, BVD-MD virus infection, cerebellum, congenital anomaly, pathology.


Many workers have described the occurrence of congenital cerebellar anomalies of calves, in which the cause was unknown or reported to be inherited [2, 10, 13, 15, 16, 18, 19, 31, 33, 35, 37]. In 1969, cerebellar hypoplasia of the calf was first reproduced by experimental infection of bovine viral diarrhea-mucosal disease (BVD-MD) virus [36]. After that, naturally-occurring congenital cerebellar anomalies have been frequently attributed to BVD-MD virus infection [1, 3–5, 20, 21, 27]. Experimental reproduction of cerebellar anomaly was also succeeded by bluetongue virus infection [24]. Aino virus is another possible factor of the disease [11]. An occurrence of cerebellar hypoplasia in the calf affected with Akabane disease is still controversial among the workers [17, 22, 23, 25, 28, 30].

The present paper describes the histopathology of congenital and perinatal cerebellar anomalies spontaneously occurred in 12 calves. It is a purpose of this study to examine the pathogenesis of cerebellar anomalies from a morphological viewpoint.

MATERIALS AND METHODS

Twelve, Holstein-Friesian or Japanese black calves of 1 to 87 days old exhibited various clinical signs soon after birth. The signs were sternal recumbency or inability to stand without support in 7 cases, blindness in 5 cases, inability to suckle without assistance in 3 cases, and domed forehead in 2 cases, as well as circling, arthrogryposis, brachycephalia and scoliosis in 1 case, respectively. Calves 1–3 were born from November to March, calves 4 and 5 in December and March, respectively, calves 6–8 from June to August, calves 9–12 from February to May.

Serum neutralizing antibodies of calves 3, 9 and 10 to Akabane and BVD-MD viruses were titrated in the sera collected after colostrum ingestion. Calf 3 had a significant level (>1,024) of neutralizing antibody to Akabane virus only, whereas calves 9 and 10 had positive levels (64 and 32, respectively) of antibodies to BVD-MD virus only. The dams of calves 9 and 10 were vaccinated
Table 1. Main pathological change in 12 calves

<table>
<thead>
<tr>
<th>Calf</th>
<th>Date of birth</th>
<th>Age (days)</th>
<th>Main pathological change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cerebellum</td>
</tr>
<tr>
<td>1</td>
<td>November</td>
<td>4</td>
<td>Heterotopia</td>
</tr>
<tr>
<td>2</td>
<td>March</td>
<td>4</td>
<td>Heterotopia</td>
</tr>
<tr>
<td>3</td>
<td>March</td>
<td>86</td>
<td>Localized loss of cortical layers</td>
</tr>
<tr>
<td>4</td>
<td>December</td>
<td>19</td>
<td>Partial defect</td>
</tr>
<tr>
<td>5</td>
<td>March</td>
<td>2</td>
<td>Partial defect</td>
</tr>
<tr>
<td>6</td>
<td>June</td>
<td>1</td>
<td>Hypoplasia</td>
</tr>
<tr>
<td>7</td>
<td>July</td>
<td>7</td>
<td>Hypoplasia</td>
</tr>
<tr>
<td>8</td>
<td>August</td>
<td>7</td>
<td>Hypoplasia</td>
</tr>
<tr>
<td>9</td>
<td>February</td>
<td>25</td>
<td>Degeneration</td>
</tr>
<tr>
<td>10</td>
<td>March</td>
<td>77</td>
<td>Degeneration</td>
</tr>
<tr>
<td>11</td>
<td>May</td>
<td>38</td>
<td>Degeneration</td>
</tr>
<tr>
<td>12</td>
<td>May</td>
<td>4</td>
<td>Degeneration</td>
</tr>
</tbody>
</table>

Fig. 1. Cerebellum of calf 1. Heterotopic cortical tissue in the white matter. HE. ×52.

against Akabane virus.

Tissues were collected systematically at necropsy, fixed in 10% neutral buffered formalin, and processed routinely. Sections from tissues embedded in paraffin-wax were stained with hematoxylin and eosin (HE). Special stains used for the central nervous system were Luxol fast blue (LFB)-HE for myelin, Bodian’s method for nerve fibers, and phosphotungstic acid hematoxylin for glial fibers.

RESULTS

Group A (calves 1–3): Calves 2 and 3 showed hydranencephaly (Table 1). Their cerebral mantles were bilaterally and incompletely lost, and variable-sized nodules of the cortical tissue attached to the inner surface of the lepto-meninx. The residual nodular tissues contained many arterioles, capillaries, and perivascular cuffs of a few hemosiderin-laden macrophages. The cerebellums of the three calves were macroscopically normal, but microscopically there were a few small nodules of heterotopic cortical tissues in the cerebellar white matter of calves 1 and 2 (Fig. 1). The external and internal granular layers and Purkinje
cells were absent in one gyrus of the cerebellar decline in calf 3.

In the spinal cords of calves 1 and 2, a decrease of nerve cells in number was prominent in the ventral horns of the cervical through lumbar cords (calf 1) or the cervical cord (calf 2). The lateral and ventral fasciculi of the cords were stained palely with LFB, associated with mild proliferation of glial fibers throughout the fasciculi. The retinas of calves 2 and 3 consisted partly of only thin layer of glial fibers (Fig. 2). The optic nerves of both cases were slightly thin, and their transverse sections revealed marked variations in fascicular caliber, loss of myelin, and mild gliosis.

The skeletal muscles of calf 1 had many hypoplastic, myoblast-like fibers in various proportions. In severely hypoplastic muscles, the interstitium was edematous and frequently infiltrated with adipose tissue. The tongue of calf 2 had similar runt muscle fibers.

**Group B (calves 4 and 5):** The cerebella of calves 4 and 5 had bilateral, semispherical concaves on the caudal surfaces of the lateral hemispheres (Fig. 3). Histologically, the concaves were margined by the white matter of the cerebellar gyrus devoid of overlying cortex. Reactive changes were absent around them. Small nodules of heterotopic cortical tissue were found in the cerebellar white matter of the cases. The calves also showed mild (calf 5) and moder-
ate (calf 4) degrees of internal hydrocephalus. The cerebral white matter of calf 4 was thin, and exhibited mild demyelination, gliosis, and formation of short splits. Chronic meningitis and hydromyelia were found at the first to third segments of the cervical spinal cord of calf 4. Spinal ganglions of the calf contained many necrotic nerve cells with pyknotic or fragmented nuclei and shrunken, palely eosinophilic cytoplasm.

Group C (calves 6–8): The cerebellums of the calves were less than one-fourth of the normal size and contained several cysts. Histologically they were characterized by cavitation of the cerebellar white matter (Fig. 4) and many microgyri (Fig. 5). The former was situated mainly in the medullary corpus and often extended into the folial white matter. The cavities often contained strips of neuropil. Gliosis and inflammatory cell infiltration were not remarkable within and around the cavity. Each microgyrus
showed a complicated pattern consisting of interlacing strands or islands of granule cells, interspersed molecular layer, and many dispersed Purkinje cells.

The brain stems of the calves were reduced in size due apparently to decreased number of nerve fibers in the cerebellar peduncles compared with age-matched controls. Two calves showed mild (calf 8) or severe (calf 7) internal hydrocephalus. In calf 7, edematous loosening of neuropil, axonal swelling, disruption of myelin, mild gliosis, and formation of a few cysts without ependymal lining were found in the compressed cerebral white matter around the lateral ventricle. The retinas of calves 6 and 7 were thin and partially replaced by strips of connective tissue. The optic nerves of the two calves were thin and on their transverse sections the fasciculi were irregular in diameter due to loss of nerve fibers and mild gliosis.

**Group D (calves 9-12):** The cerebellums of the calves were macroscopically intact or slightly shrunken, but microscopically they revealed marked degenerative changes without malformation.

The cerebellum of calf 9 was slightly shrunken and gyration of the dorsal surface was flattened. Microscopically, the cortex was reduced in thickness as it approached to the meninx. The outermost layer of the cortex was sometimes replaced by glial fibers or lost completely exposing the underlying folial white matter directly to the meninx. The moderately atrophic cortex was scarce in granule and Purkinje cells, having some torpedoes in the granular layer. The external granular layer was atrophied to one- to two-cell thick and mitotic figures were occasionally observed in the external granule cells. The cerebrum of the calf showed severe internal hyd-
rocephalus and contained a worm of *Setaria* in a dilated lateral ventricle. The white matter of the cerebral hemispheres was extremely thin and loose with loss of nerve fibers, cavitation, and gliosis. Parts of the cerebral cortex in the parietal, temporal, and occipital lobes were liquefied or diffusely gliosed (Fig. 6). Calcium corpuscles were scattered in the grey and white matters of the cerebrum. Many macrophages and giant cells infiltrated around the worm. The third ventricle and aqueduct were slightly contracted and irregularly contoured without ependymal lining (Fig. 7). Glial fibers proliferated densely around the channels. Slits, cavities, tubules with ependymal lining, and clusters and rosettes of ependymal cells were frequently formed within the glial tissue. The fourth ventricle and central canal of the medulla oblongata were also devoid of ependymal lining, but the central canal of the spinal cord was intact. The white matter of the spinal cord was diffusely degenerated with a mild degree of gliosis and an infiltration of a few macrophages.

The cerebellum of calf 10 appeared normal at necropsy, but close macroscopical examination revealed a mild shrinkage of individual gyri. Microscopically, the entire cortex of the cerebellum was about one half to one-third the thickness of the age-matched control. The molecular and granular layers, and folial white matter were proportionally thin (Fig. 8). Purkinje cells were occasionally dislocated into the molecular layer, degenerated with cytoplasmic vacuoles, atrophied with pyknotic nuclei, and disappeared leaving empty baskets. Necrotic granule cells and torpedoes preferably appeared in the superficial region of the granular layer.

The cerebellar cortex of calf 11 was thin due to thinning of molecular layer, and a decrease in granule and Purkinje cells at
gyri of the paleocerebellum. Pyknotic granule cells and torpedoes were frequent in the internal granular layer of the gyri. The cerebrum of the calf was slightly shrunken and firm with dilated sulci and many air bubbles beneath the meninx. The lateral ventricles were slightly dilated. Microscopically, the neopallium and cerebral white matter were thin in variable degrees depending upon gyri and locations. Laminar structure of the affected cortex was indiscernible due to widespread loss of nerve cells and fibrillary gliosis (Fig. 9). Neurons in the second and fourth layers of the cerebral cortex were relatively better preserved than those in the other layers. Residual nerve cells were shrunken and oriented in random directions. The periventricular white matter was diffusely demyelinated and contained many fibrillary and gemistocytic astrocytes. The paleopallium and basal nuclei were relatively spared except the Sommer’s sector in which a row of pyramidal cells was interrupted by glial tissues (Fig. 10). Vascular walls were intact throughout the brain and arachnoid membrane of the cerebral hemispheres was slightly thickened. Many nerve cells of the olivary nucleus were dark and shrunken with pyknotic nuclei, and the white matter of the brain stem was occasionally gliosed.

The cerebellum of calf 12 was slightly small with normal proportion, and the cortex in the severely affected gyri was less than a half the thickness of the age-matched control. Microscopically, granule and Purkinje cells were markedly decreased in number, and the latter frequently showed degenerative changes such as vacuolation and chromatolysis of cytoplasm with eccentric nuclei. The molecular layer was thin and slightly hypercellular containing neuroblasts and a few dislocated Purkinje cells. Small, patchy areas of gliosis were scattered in the white matter of the cerebellum. The lateral ventricles of the calf were slightly dilated and on LFB-HE sections there was a slight decrease of myelin in the periventricular white matter.

DISCUSSION

The small-sized cerebellums of neonatal calves have collectively been referred to as hypoplasia irrespective of morphological details in the cerebellar anomalies [1, 4, 13, 15, 27, 31, 33, 38]. Whereas, some workers preferred the designation, cerebellar atrophy, rather than hypoplasia [8]. In the present study, cerebellar anomalies of 12 calves were divided morphologically into four groups: the macroscopically normal cerebellums with minor histological malformations (group A); partial defect of the lateral hemispheres without degenerative and reactive changes (group B); severe, proportional gross reduction characterized histologically by microgyria and cavitation in the white matter (group C); and mild gross reduction due to degeneration with a slight degree of gliosis in the cortex (group D). Heterotopia and microgyria found in groups A-C were dystopic changes and might have been caused by inhibition of neuroblastic migration [32, 34]. The critical period for the development of the changes terminates at the fifth month of fetal life in humans [32] when lamination of the cerebellar cortex is in progress [14]. On the other hand, neuroglia first appears in the cerebellum later than the third month of gestation [29], and astrocytic reaction becomes more conspicuous during later gestation stages [26] when lamination of the cerebellar cortex has been completed. Therefore, it seems appropriate that cerebellar changes in group C are referred to as hypoplasia and those in group D as degeneration. The designation of cerebellar atrophy was not applied to group D since a reduction of the cerebellar size was not macroscopically obvious in two of the four calves examined.
Runt muscle fibers found in calves 1 and 2 are a characteristic change of Akabane disease [22, 23]. Calf 3 showed hydranencephaly and a significantly high level of neutralizing antibody to Akabane virus. A total of 71 calves suspected of Akabane disease was necropsied during the past 13 years in our Department, of which only the present three calves showed cerebellar abnormalities. These results might indicate a rare occurrence of minimal cerebellar malformations in calves affected with Akabane disease. Blindness which occasionally appears in calves affected with Akabane disease [17, 22, 23, 25, 30, 37] might be attributable for some cases to atrophy of the retina and optic nerve as well as hydranencephaly, since two of three calves in group A showed optic lesions.

The precise morphological analogues of cerebellar malformations in group B could not be found in literature. The cause(s) of the cerebellar changes as well as chronic meningitis (calf 4) and multiple anomalies (calf 5) were poorly defined in the present study.

Cerebellar changes in group C concurred well with those in the calves experimentally infected with BVD-MD virus [8, 9, 12]. Retinal atrophy found in two of three calves of group C is a usual complication of cerebellar hypoplasia in BVD-MD virus infection [6, 7, 21, 36]. Therefore, cerebellar lesions of the three calves might be evolved by BVD-MD virus infection. Although internal hydrocephalus has rarely been induced by experimental infection of BVD-MD virus [6–9, 12], naturally-occurring cerebellar hypoplasia suspected of BVD-MD virus infection frequently accompanies the cerebral lesion [1, 4, 27, 38]. The pathogenesis of the cerebral lesion in naturally-occurring BVD-MD virus infection is still unknown.

Neutralizing antibody to Akabane virus was negative in calves 9 and 10. Calves 11 and 12 were delivered at term on May when Akabane disease does not occur [22, 23]. Therefore, an involvement of the disease was unlikely in four calves of group D. The cerebrum of calf 9 revealed severe, progressive internal hydrocephalus with dysplasia of the third ventricle and aqueduct. Cerebellar changes of the calf occurred predominantly at marginal areas of the cerebellum and were considered to be caused chiefly by increased intracranial pressure. The significance of a worm of Setaria aberrantly migrated into the lateral ventricle for the development of dysplastic ventricles was inconclusive.

Cerebral changes in calf 11 are ulegria which, to our knowledge, no case reports have been in veterinary literature, despite the presence of descriptions in many books. However, the change is well known in human pathology as sequelae of hypoxia during a perinatal period [34]. Degenerative changes in the paleocerebellar cortex and brain stem including the olivary nucleus seem to be complications of ulegria.

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REFERENCES


先天性および周産期小脳異常子牛12例の病理学組織像：梅村孝司・佐藤宏・御領政信・板倉哲敏（鳥取大学農学部家畜病理学教室）——12例の子牛に見られた先天性および周産期小脳異常を形態学的に型に分類した。
12例中3例は皮質組織の異所着色あるいは1脳回に限局した頭顔および脳室管細胞層の欠損などの軽微な組織異常を示し、アカバネ病と診断された。他の2例では左右小脳半球における部分的な対称性組織欠損がみられた。別の3例および4例は、それぞれ小脳皮質および小脳変性を示し、小脳変性はウイルス性下痢症—粘膜
病ウイルス感染によるものと考えられたが、小脳変性の病理発生は一様ではなく、周産期下痢症あるいは小脳変
異常性に基づく頭蓋内圧亢進によって惹起された症例が含まれるものと思われた。