

Biochemical analysis revealed an increase in G_{M1} in the fetal brain^{383, 386, 392} and β-galactosidase deficiency in liver or brain.^{381, 383} An affected fetus was aborted with intrauterine hypertonic saline instillation.³⁸³ Fetal tissues were adequate for subsequent biochemical diagnosis.

ANIMAL MODELS

Animal models of G_{M1} gangliosidosis have been recorded in cats,³⁹³⁻³⁹⁹ dogs,⁴⁰⁰⁻⁴⁰³ sheep,⁴⁰⁴⁻⁴⁰⁷ and calves.⁴⁰⁸⁻⁴¹⁰ They are domestic animals with spontaneously occurring storage diseases. Although inbred strains of laboratory rodents had not been reported, mouse models of G_{M1} gangliosidosis have been created recently through targeted disruption of the β-galactosidase gene.⁴¹¹⁻⁴¹³ All the model animals show central nervous system manifestations, with a specific deficiency of β-galactosidase.

Feline G_{M1} Gangliosidosis

A neurologic disorder with clinical, morphologic, biochemical, and genetic similarities to human G_{M1} gangliosidosis has been described in two families of Siamese cats,^{359, 393, 394, 397, 398, 414, 415} in two families of short-haired domestic cats,^{189, 395, 396, 416} and in a Korat cat.³⁹⁹ Affected kittens appear normal at birth, and tremors of the head and hind limbs are first noted at 2 to 3 months of age, followed by generalized dysmetria and spastic quadriplegia. Affected cats show exaggerated acousticomotor response, impaired vision, and generalized convulsive seizures by 1 year of age. This disease is transmitted as an autosomal recessive trait.

A 7-month-old Korat cat was referred for a slowly progressive neurologic disease.³⁹⁹ β-Galactosidase activity was very low in peripheral leukocytes, brain, and liver. Histologically, diffuse vacuolization and enlargement of neurons were observed throughout the brain, spinal cord, and peripheral ganglia, and there was severe cerebellar neuronal cell loss with moderate astrocytosis. Ultrastructurally, neuronal vacuoles were filled with concentrically whorled lamellae and small membrane-bound vesicles. A striking increase in G_{M1} was found in the brain. D. Maria and colleagues concluded that clinical onset, morphologic and histochemical features, and biochemical findings in the Korat cat G_{M1} gangliosidosis were comparable with those in the human juvenile form, although the clinical course, survival time, and residual enzyme activity level did not completely fit those of the human disease.

A widespread neuronal vacuolation is observed microscopically in brain, retina, spinal cord, and peripheral ganglia. Numerous MCBs are observed electron microscopically, with multiple concentric lamellae simulating those in the human disease. Lesions outside the nervous system are limited to hepatocellular vacuolation and aspermatogenesis.

Neutral oligosaccharides are markedly increased in the tissues and urine of affected cats.¹⁸⁹ The core structure of a hexasaccharide has been identified as Galβ-GlcNAc-Man-Man-GlcNAc with an additional *N*-acetylglucosamine.^{188, 190} Four major types of oligosaccharides were proposed by Barker and colleagues.¹⁸⁹ Their structures suggest that they arise from incomplete catabolism of *N*-glycans of glycoproteins.

A family of Siamese cats^{359, 393, 414, 415} that was extensively investigated clinically and biochemically is linked to late infantile/juvenile G_{M1} gangliosidosis in humans.^{190, 396, 417} The cats developed normally until the age of 4 months, and then neurologic manifestations followed. Physical appearance was normal, and no hepatosplenomegaly was observed. Electrodiagnostic tests revealed that motor and sensory nerve

conduction velocities remained within normal limits, but spinal-evoked potentials indicated slowing in conduction velocity, and brain stem auditory-evoked responses indicated prolonged latencies.⁴¹⁸ Some residual enzyme activity of β -galactosidase was detected in brain (15 percent of normal).³⁹³ β -Galactosidase deficiency has been demonstrated in brain, kidney, liver, spleen, skin, cultured fibroblasts, and cultured conjunctival cells⁴¹⁹ associated with remarkable accumulation of ganglioside G_{M1} . The mutant enzyme has a reduced K_m for 4-methylumbelliferyl β -galactoside, high K_m for G_{M1} and asialofetuin, increased thermostability, and a shift of isoelectric point.⁴¹⁷

Several investigations into the pathogenesis of neuronal dysfunction have been performed using this feline model of human disease. These showed (1) increased gangliosides, cholesterol, and phospholipids in synaptosomal membranes,³⁵⁴ (2) reduced fluidity of synaptosomal membranes,³⁵⁴ (3) meganeurites between the soma and initial axon segment and aberrant secondary neurites,^{348, 349} suggesting a possible role for gangliosides in neuronal differentiation and synaptogenesis,^{346, 348} (4) a gradient in total ganglioside and G_{M1} concentration and the proportion of docosanoate (22:0) in G_{M1} , corresponding to the morphologic gradient of meganeurite formation (cerebral cortex > caudate = thalamus > cerebellum),³⁵³ (5) increased acetylcholine synthesis and release in cerebral cortex and hippocampal slices,^{358, 359} (6) a decrease in high-affinity uptake of glutamate, g-aminobutyrate, and norepinephrine in synaptosomes,³⁶¹ (7) alteration of phospholipase C and adenyl cyclase activities,³⁶³ (8) alteration of evoked synaptic activity patterns in cortical pyramidal neurons,⁴²⁰ (9) reduced calcium flux in synaptosomes,⁴²¹ (10) quantitatively and qualitatively normal phorbol ester receptors in the brain,⁴²² and (11) common occurrence of glutamic acid decarboxylase-immunoreactive spheroids in many brain regions, whereas limited in distribution in other diseases.³⁶² These data suggest that various morphologic and metabolic aberrations occur in the presence of excessive storage of ganglioside G_{M1} .

Cox and colleagues³⁹⁷ observed that thymuses from affected cats older than 7 months of age (G_{M1} mutant cats) show marked thymic reduction in size (approximately threefold) compared with age-matched normal cats. Histologic sections of lymph nodes, adrenal glands, and spleens showed no significant differences. Flow cytometric analysis showed a marked decrease in the percentage of immature CD4+CD8+ thymocytes and an increase of CD4-CD8+ cells in mutant cats. Colabeling with CD4, CD8, and CD5 indicated an increase in the percentage of CD5-high thymocytes, suggesting the presence of more mature cells. In peripheral lymphocytes there was an increase in CD4-CD8+ cells and a slight decrease in CD4+CD8- and CD4-CD8- cells. Cats younger than seven months showed no abnormalities in thymocyte or lymphocyte subpopulations. However, surface G_{M1} expression of thymocytes detected by cholera toxin B binding was increased at all ages in affected cats. Also, in situ labeling for apoptosis was increased between 3 and 7 months of age when thymic masses were within normal limits. After 7 months, the labeling was decreased. These data indicate premature thymic involution in feline G_{M1} gangliosidosis and suggest that increased surface G_{M1} alters thymocyte development in these cats.

In another experiment, incorporation of exogenous G_{M1} into feline thymocyte cell membranes produced a dose-dependent increase of apoptotic cell death.⁴²³ Although CD4 expression on both feline thymocyte and lymph node cell membranes was abruptly decreased after introducing exogenous G_{M1} , enhanced apoptotic death was observed only in thymocytes and not in lymph node cells at the same G_{M1} concentration. Enhancement of thymocyte apoptosis appears to be age-related; the cells from cats younger than 3 months were more vulnerable.

Growth hormone (GH) and insulin-like growth factor I (IGF-I) are important hormonal peptides supporting thymic function and affecting body growth, particularly in the prepubescent period. In GM₁ gangliosidosis cats, serum IGF-I was low by 5 months of age, and a peripubertal increase was not observed.³⁹⁸ In addition, IGF-binding protein 3 was reduced and IGF-binding protein 2 was elevated after 7 months. mRNA for hepatic IGF-I and pituitary GH was significantly reduced. Stimulation by exogenous recombinant canine GH elevated serum IGF-I. These results suggested that GH/IGF-I signaling pathways in the liver remain intact and that alterations are external to the liver.

Canine GM₁ Gangliosidosis

The canine disease has been described in mixed-breed beagle dogs,⁴⁰⁰ English springer spaniels,^{402, 424, 425} Portuguese water dogs,^{403, 426} Alaskan huskies,^{427, 428} and Shiba dogs.^{429, 430} Two canine models, the English springer spaniel and the Portuguese water dog, have been compared.⁴²⁴ The clinical course and severity of skeletal dysplasia and progressive neurologic impairment were similar in these models. The skeletal lesions are similar to those in a child with GM₁ gangliosidosis.⁴³¹ However, dwarfism and coarse facial features were seen only in English springer spaniels; glycoproteins containing polylactosaminoglycans were found in visceral organs. Portuguese water dogs did not show these clinical or biochemical changes. It seems difficult and inappropriate to apply clinical classification of GM₁ gangliosidosis in humans to these model animals.⁴²⁴ Dysostosis multiplex, dwarfism, and orbital hypertelorism with coarse facial features were found in the cases reported by Alroy and colleagues,⁴⁰² probably representing a model of infantile GM₁ gangliosidosis.

Pathologic and biochemical changes are similar to those in the feline cases described earlier.⁴³² The ultrastructural and biochemical features suggested that the disease in the first reported dog had features similar to both the infantile and juvenile forms of human GM₁ gangliosidosis (see Table 151-1).⁴⁰¹ Serial MRI studies revealed an abnormal signal intensity of cerebral and cerebellar white matter observed on T₂-weighted images.⁴³² Some biomarkers, including aspartate aminotransferase, lactate dehydrogenase, creatine kinase, neuron-specific enolase, and myelin basic protein, as well as ganglioside GM₁, were significantly higher in the CSF of GM₁ gangliosidosis dogs than of control dogs, and their changes were well related to the age and clinical course.⁴³³ These biomarkers may be used for monitoring the effect of therapeutic trials in the near future.

Analysis of the major oligosaccharides accumulating in the dog revealed that the compounds are nearly identical to the oligosaccharides stored in human GM₁ gangliosidosis liver, but they differ from the human compounds uniquely because they contain two GlcNAc residues at the reducing terminus instead of one, suggesting that there may be significant differences in glycoprotein metabolism or structure between mammalian species.¹⁸⁸ Kinetic properties of the mutant enzyme in affected dogs were identical to those from normal dogs, but the amount of CRM was markedly reduced.³⁸⁸

Antiserum raised against purified human liver β-galactosidase crossreacted with the enzyme from dog liver but not with those from cat liver or *Escherichia coli*.⁴³⁴ Furthermore, 21 of the 24 tryptic peptides of the dog β-galactosidase were homologous with those of the human enzyme. A partial canine β-galactosidase cDNA was 86 percent homologous to the human counterpart, and preliminary analysis of a genomic library indicated conservation of exon number and size.⁴³⁵

The acid β -galactosidase cDNA of Portuguese water dogs was isolated and sequenced.⁴²⁵ The entire coding region of the gene consists of 2004 nucleotides encoding a protein of 668 amino acids—approximately 86.5 percent identity at the nucleotide level and about 81 percent identity at the amino acid level with the human acid β -galactosidase gene. The deduced amino acid sequence contains a 24-amino-acid putative signal sequence, six possible glycosylation sites, and seven cysteine residues. A homozygous recessive mutation causing canine G_{M1} gangliosidosis was identified at nucleotide G200→A in exon 2, resulting in an amino acid substitution Arg60→His (R60H). The mutation creates a new restriction site for Pml1. Genotyping 115 dog samples for this gene mutation revealed 5 affected homozygotes, 50 heterozygous carriers, and 60 normal homozygotes. Based on these observations, it was concluded that dog and human β -galactosidases are structurally similar and that canine G_{M1} gangliosidosis is an excellent model for the human disease.

Retarded endochondral ossification and osteoporosis were observed at 2 months of age in canine G_{M1} gangliosidosis and focal cartilage necrosis within lumbar vertebral epiphyses in older puppies.⁴³¹ The lesions were characterized histologically by chondrocytic hypertrophy and lysosomal accumulation of storage materials. These changes were similar to those in a human child with G_{M1} gangliosidosis. Chondrocytes from affected dogs cultured in agarose contained numerous large vacuoles and had a reduction in mitosis and Alcian blue–staining proteoglycans.⁴³⁶ This culture system may be a useful method for studying the biology of cartilage leading to skeletal abnormalities in lysosomal storage diseases.

Ovine G_{M1} Gangliosidosis

An inherited disease associated with deficiencies of β -galactosidase (5 percent normal) and α -neuraminidase (20 percent normal) has been observed in sheep.^{407, 437} The neuraminidase activity was not restored after addition of leupeptin in the culture medium of fibroblasts.^{404, 405} Neuronal storage was much more extensive than visceral storage. Kupffer cells and macrophages from bone marrow were affected similarly to but less severely than hepatocytes and renal epithelial cells, whereas hematopoietic cells and chondrocytes were unaffected.⁴³⁷ Results of lectin histochemistry suggested that the stored material in this disease has terminal saccharide moieties consisting of β -galactose, *N*-acetylneuraminic acid, and *N*-acetylgalactosamine.⁴³⁸ An interspecific genetic complementation analysis indicated that the ovine β -galactosidase deficiency is due to a mutation at the genetic locus homologous with that of human G_{M1} gangliosidosis and Morquio B disease in humans.⁴³⁹ A genetic defect in lysosomal β -galactosidase may cause the deficiency of lysosomal α -neuraminidase in sheep.^{404, 440}

The initial clinical sign of ovine G_{M1} gangliosidosis is ataxia around 5 months of age, and neurologic deterioration follows^{404, 405}: ataxia, conscious proprioceptive deficit most severe in the hind limbs, blindness, and recumbency.⁴⁴¹ An ovine fetus affected with G_{M1} gangliosidosis at 4 months of gestation (normal gestation 5 months) showed marked cytoplasmic enlargement and vacuolation of central and peripheral nervous system neuronal soma and of hepatocytes and renal epithelial cells.⁴⁴² This case indicates the need for prenatal initiation of therapy. Vacuolization seems to be not the single cause of clinical signs in the ovine disease because the clinical signs do not commence until at least 5 months after vacuolization is apparent histologically.

Another form of ovine G_{M1} gangliosidosis was reported recently.⁴⁰⁶ Neurologic signs were observed at approximately 1 month of age. A specific deficiency of β -galactosidase was found, with relatively high residual activity (10 percent) compared with that described earlier. Neuraminidase activity was normal. Storage of G_{M1} and asialo-G_{M1} was not reported for this case. In both ovine cases, molecular genetics

analysis has not been performed.

During a large neuropathologic survey of clinically normal sheep for evidence of scrapie, three adult Romney ewes from the same farm were found to have populations of distended neurons containing granular eosinophilic storage material.⁴⁴³ The affected neurons were confined to the striatum. The granules had the tinctorial properties of glycolipid and the ultrastructural appearance of spherical bodies containing concentric membranous whorls. The bodies resembled those of GM₁ gangliosidosis. The restricted neuroanatomic distribution of changes, together with the age of the sheep, suggests a similarity to human type 3 GM₁ gangliosidosis. However, no biochemical evidence has been provided for solid diagnosis of the disease.

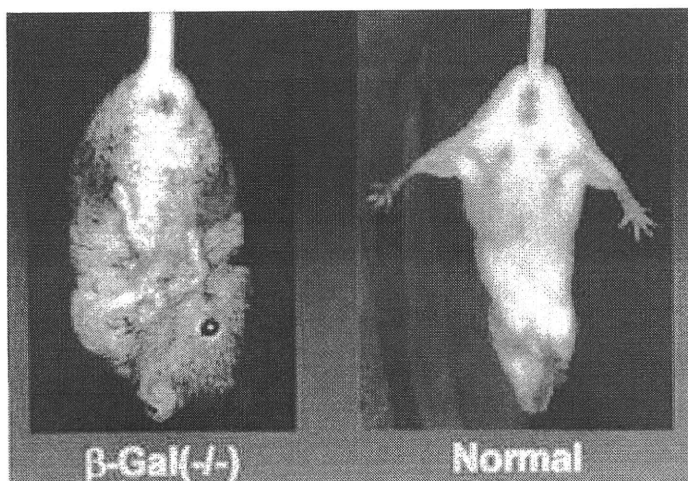
Bovine GM₁ Gangliosidosis

Pathologic, biochemical, and enzymatic studies were performed in Friesian calves with GM₁ gangliosidosis. GM₁ accumulated in neurons, and β -galactosidase deficiency was confirmed.⁴⁰⁸⁻⁴¹⁰ However, morphologic hepatic changes were minimal or absent, without significant elevation of mucopolysaccharides.⁴⁴⁴ Vision is impaired at the late stage of the disease. The ocular lesions were confined to the retina and optic nerves.⁴⁴⁵ Retinal ganglion cells and amacrine cells in the inner nuclear layer are distended with dense aggregates of MCBs. Wallerian degeneration is present in the optic nerves.

Murine GM₁ Gangliosidosis Generated by Gene Targeting (Knockout Mouse)

Gene targeting in embryonic stem cells generated mouse models⁴¹¹⁻⁴¹³ for conventional laboratory use. Homozygous mutants were born normally and apparently healthy until 4 months of age, although poor achievement on the water maze test was observed in some mutant mice after 2 months. Then horizontal movement became slow, and rearing or vertical climbing became less frequent. By 6 to 8 months, definite gait disturbance with mild shaking was observed, and spastic diplegia progressed. When hung vertically with the tail held upward, the mice huddled themselves with all four limbs flexed (Fig. 151-12). Normal mice always extended their limbs in the downward vertical direction. At the terminal stage, generalized and progressive paralysis appeared, and the affected mice died of extreme emaciation at 7 to 10 months of age. Hepatosplenomegaly or skeletal dysplasia is not detected. About half the homozygote pairs could produce at least one litter; thus the knockout mice could be maintained as homozygous mutants.⁴¹²

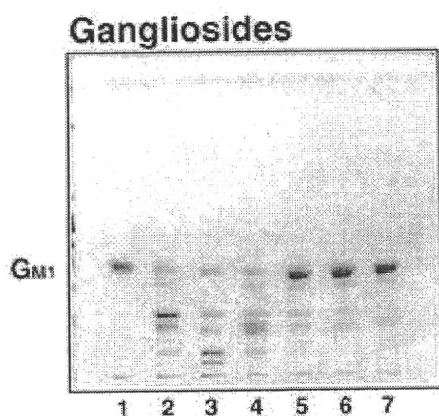
Fig. 151-12:



Abnormal posture of a β -galactosidase-deficient mouse in the downward vertical position. A, An affected β -Gal(-/-) mouse (8 months old) huddles himself with all limbs flexed and paws clenched. B, A normal β -Gal(+/+) littermate with normal reaction. (Reproduced from Matsuda et al. 411 Used by permission.)

Three biochemical markers confirmed that the affected mice are authentic models of human GM1 gangliosidosis: generalized β -galactosidase deficiency, abnormal storage of GM1 in brain and other tissues (Fig. 151-13), and hyperexcretion of urinary oligosaccharides.

Fig. 151-13:



Thin-layer chromatographic pattern of mouse brain gangliosides. Resorcinol stain. Lane 1 = GM1 (reference standard); 2 = cerebrum (wild type); 3 = brain stem (wild type); 4 = cerebellum (wild type); 5 = cerebrum (knockout); 6 = brain stem (knockout); 7 = cerebellum (knockout). A remarkable increase in GM1 is observed in three different areas of the knockout mouse. (Reproduced from Matsuda et al. 412)

Used by permission.)

In a pathologic study,⁴⁴⁶ vacuolated neurons appeared in the spinal cord 3 days after birth. The vacuolation extended to neurons in the brain stem, cerebral cortex, hippocampus, and thalamus, and ballooning neurons became prominent with age. The vacuolation also appeared in Purkinje cells without marked ballooning. Reactive astrogliosis was prominent in the entire brain at the terminal stage of the disease. Immunohistochemical study using anti-G_{M1} and anti-G_{A1} antibodies revealed extensive accumulation of G_{M1} and G_{A1} in the cerebral neurons. In the liver, however, accumulation of G_{M1} was localized to the cytoplasm of hepatocytes, whereas that of G_{A1} was localized to foamy macrophages and Kupffer cells. There were no significant abnormalities in the bone, bone marrow, or cornea at any stage. HPTLC analysis showed that the G_{M1} content of the mutant mouse brain was not pathologically increased at birth. An increase in G_{M1} and asialo-G_{M1} occurred rapidly to the same degree of storage. This remarkable accumulation of asialo-G_{M1} may be explained by a metabolic turnover of G_{M1} to asialo-G_{M1} catalyzed by a specific neuraminidase in mice. A recent report has confirmed that intact and living cells of a neurotumoral nature express a neuraminidase that hydrolyzes ganglioside G_{M1} and G_{M2}.⁴⁴⁷ This degradative process seems to occur in lysosomes because it is blocked by the conditions preventing endocytosis or inhibiting lysosomal enzyme activities.

Skeletal dysplasia is observed in human patients with infantile G_{M1} gangliosidosis and Morquio B disease but not in mutant mice. Urinary mucopolysaccharides were normal in amount and pattern in the mutant mice. The mouse does not synthesize keratan sulfate in the skeletal system.⁴⁴⁸ In another report, the mouse rib cartilage contained only small amounts of keratan sulfate of extremely small size.⁴⁴⁹ This may explain an apparent lack of skeletal dysplasia in mutant mice, although more time may be necessary for development of generalized skeletal dysplasias.⁴¹²

We further produced a transgenic (Tg) mouse line overexpressing mutant human β -galactosidase with an amino acid substitution (R201C) that causes juvenile type G_{M1} gangliosidosis in humans by injecting the DNA fragment containing β -actin promoter (CAG promoter) and human mutant β -galactosidase cDNA (R201C) into C57BL/6 fertilized eggs. Cross-breeding of the KO mice and Tg mice was performed to obtain KO-Tg mice (R201C mice). We used hemizygous Tg mice with the β -galactosidase KO background for the study described earlier in the experiments of chemical chaperone therapy.³³⁹ The R201C mice, expressing the human R201C mutant but lacking the endogenous mouse β -galactosidase, had very low β -galactosidase activity in the brain (~4 percent of the wild-type activity). They exhibited an apparently normal clinical course for the first 6 months after birth, and this was followed by slowly progressive neurologic deterioration, such as tremor and gait disturbance, during the next 9 months. Death ensued around 15 months of age owing to malnutrition and emaciation. Neuropathologic examination revealed vacuolated or ballooned neurons, but they were less abundant than those in the KO mouse brain. Intracytoplasmic storage materials were present in pyramidal neurons and brain stem motor neurons but not in neurons in the other areas of the brain. There was no storage in glial cells.

TREATMENT

Therapeutic experiments using cultured cells have been reported to enhance the uptake of the exogenous enzyme and to prolong its intracellular half-life. A thiol protease inhibitor prolonged the effect of exogenous β -galactosidase in human G_{M1} gangliosidosis fibroblasts.²⁵⁸ Enzyme replacement of cultured cells from cats with G_{M1} gangliosidosis was tried with the liposome-entrapped enzyme, and the storage of glycopeptides decreased.⁴⁵⁰

Allogeneic bone marrow transplantation was performed in an 81-day-old Portuguese water dog affected with GM1 gangliosidosis using a dog leukocyte antigen-identical sibling as donor.⁴⁵⁰ Complete engraftment was achieved, and β -galactosidase activity in leukocytes of the transplanted dog was similar to that in the donor. However, neither the subsequent clinical course nor the enzyme activity was modified. It was concluded that bone marrow transplantation early in life is ineffective in canine GM1 gangliosidosis.

Amniotic tissue transplantation was tried on a patient with Morquio B disease.⁴⁵¹ No clinical improvement was observed. Only conservative therapy has been performed for human GM1 gangliosidosis patients. Dystonia was markedly improved dose-dependently by oral administration of trihexyphenidyl in an adult GM1 gangliosidosis patient.⁴⁵² No side effects were observed. The extrapyramidal signs in adults with GM1 gangliosidosis are probably caused by hypofunction of dopaminergic neurons or relative hyperactivity of cholinergic neurons in the basal ganglia.

In our experiment with adenovirus-mediated gene transfer to newborn knockout mice, β -galactosidase increased to 10 to 20 percent of normal activity in the brain for 60 days, and the GM1 storage was significantly prevented. We concluded that neonatal administration via blood vessels provided access to the central nervous system because of the incompletely formed blood-brain barrier.⁴⁵³

Chemical Chaperone Therapy

In recent years, we have tried a new molecular therapeutic approach to lysosomal diseases. Some mutant enzyme proteins were labile and rapidly degraded in somatic cells from patients with Fabry disease with genetic α -galactosidase A deficiency.^{454, 455} Their catalytic activities were stabilized and restored in culture cells by addition of galactose⁴⁵⁶ or 1-deoxygalactonojirimycin⁴⁵⁷ in the culture medium. A short-term galactose infusion therapy was found to be clinically effective in a Fabry patient.⁴⁵⁸ This paradoxical phenomenon was confirmed also for β -mutant galactosidasae with 1-deoxygalactonojirimycin.⁴⁵⁹ In addition, newly synthesized in vitro enzyme inhibitors *N*-octyl-4-*epi*- β -valienamine (NOEV) and *N*-octyl- β -valienamine (NOV) remarkably restored mutant enzyme activities in cultured fibroblasts from some patients with β -galactosidase and β -glucosidase deficiency disorders (GM1 gangliosidosis and Gaucher disease), respectively.^{339, 460} They are actually specific competitive inhibitors of respective lysosomal enzymes in vitro, and they serve as stabilizers of mutant proteins in the cell (chemical chaperone).⁴⁶¹⁻⁴⁶³

The Tg mouse expressing the mutant β -galactosidase gene (R201C) causing juvenile GM1 gangliosidosis was fed orally with NOEV water solution for a week. All tissues, including the central nervous system, showed a marked increase in enzyme activity.³³⁹ Immunohistochemical stain revealed a decrease in the amount of GM1 and GA1 in neuronal cells in the frontotemporal cerebral cortex and brain stem. Subsequently, we confirmed that the chaperone NOEV, after oral administration and intestinal absorption into the bloodstream, reached the mouse brain through the blood-brain barrier and enhanced the mutant β -galactosidase activity, resulting in substrate digestion and clinical improvement.⁴⁶⁴ No specific adverse effects have been observed for at least 6 months of continuous oral administration in experimental mice.

This is the first achievement for developing a new therapeutic approach by oral medication directly and specifically targeting the brain pathology in lysosomal storage diseases. This new molecular therapy (chemical chaperone therapy) will be useful for certain patients with β -galactosidosis and potentially other lysosomal storage diseases with central nervous system involvement.³³⁹

A clinical survey using cultured fibroblasts for selection of patients for this approach indicated that NOEV chaperone therapy will be effective in 20 to 40 percent of the patients, particularly in juvenile and infantile GM₁ gangliosidosis patients.³³⁸ The effect is mutation-specific. Most effective mutations are R201C, R201H, and R457Q. NOEV treatment at the early stage of the disease resulted in an arrest of disease progression within a few months in R201C mice⁴⁶⁴ based on a newly developed clinical assessment method for monitoring brain damage in experimental mice.⁴⁶⁵

NOV is the second compound for chemical chaperone therapy in our study. Apparently deficient β-glucosidase activity was enhanced by NOV in some Gaucher fibroblasts.^{460, 466} Similar therapeutic approaches have been reported recently from other laboratories for Pompe disease using deoxynojirimycin and *N*-butyldeoxynojirimycin^{467, 468} and for GM₂ gangliosidosis using pyrimethamine⁴⁶⁹ as promising chaperones. We expect that they also will serve as new chaperone drugs for these diseases.

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