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Research Paper

The Effect of Interleukin-1 Antagonists on Brain Volume and Cognitive Function in Two Patients With Megalencephalic Leukoencephalopathy With Subcortical Cysts

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ABSTRACT

Background: Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a rare leukodystrophy characterized by early-onset macrocephaly and progressive white matter vacuolation. The MLC1 protein plays a role in astrocyte activation during neuroinflammation and regulates volume decrease following astrocyte osmotic swelling. Loss of MLC1 function activates interleukin (IL)-1β-induced inflammatory signals. Theoretically, IL-1 antagonists (such as anakinra and canakinumab) can slow the progression of MLC. Herein, we present two boys from different families who had MLC due to biallelic *MLC1* gene mutations and were treated with the anti-IL-1 drug anakinra.
Methods: Two boys from different families presented with megalencephaly and psychomotor retardation. Brain magnetic resonance imaging findings in both patients were compatible with the diagnosis of MLC. The diagnosis of MLC was confirmed via Sanger analysis of the *MLC1* gene. Anakinra was administered to both patients. Volumetric brain studies and psychometric evaluations were performed before and after anakinra treatment.
Results: After anakinra therapy, brain volume in both patients decreased significantly and cognitive functions and social interactions improved. No adverse effects were observed during anakinra therapy.
Conclusions: Anakinra or other IL-1 antagonists can be used to suppress disease activity in patients with MLC; however, the present findings need to be confirmed via additional research.

Introduction

Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a rare genetic disorder characterized by early-onset macrocephaly and progressive white matter vacuolation.[1,2] Patients with MLC predominantly present with motor developmental delay, seizures, and macrocephaly; however, extrapyramidal symptoms and ataxia can also occur.[3] The disease primarily results from recessive mutations in the *MLC1* gene and recessive or dominant mutations in the *HEPACAM* (also called *GLIALCAM*) gene. Patients carrying biallelic *MLC1* or *HEPACAM* mutations usually present with a classical phenotype characterized by ataxia, spasticity, and late-onset mild mental deterioration, whereas dominant mutations in *HEPACAM* result in a less severe phenotype characterized by amelioration of motor function, with or without autism.[3]

The diagnosis of MLC is based on clinical and imaging findings and can be confirmed via genetic studies. Brain magnetic resonance imaging (MRI) in patients with MLC shows a diffuse signal abnormality, swelling of the cerebral white matter, and subcortical cysts that are primarily present in the anterior temporal areas and the frontoparietal region. To date, there is no definitive treatment for the disease, and supportive therapies such as antiseizure medications and physical therapy are usually recommended. MLC1 protein (MLC1) is a membrane protein expressed by the perivascular

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astrocytes that plays a central role in astrocyte activation during neuroinflammation and regulates volume decrease after astrocyte osmotic swelling. Loss of MLC1 function activates interleukin (IL)-1β-induced inflammatory signals4[4]; therefore, theoretically, IL-1 antagonists (such as anakinra [ANA] and canakinumab) can slow the progression of MLC. Herein, we present two boys from different families who had MLC due to biallelic *MLC1* gene mutations and were treated with the anti-IL-1 drug ANA. ANA has been used for the treatment of some diseases affecting the central nervous system (CNS) such as neonatal-onset multisystem inflammatory disease and autoimmune-related seizures. However, there has not yet been a case of MLC treated with ANA. The dose of ANA given to the patients was planned based on previous studies of ANA on CNS disorders.

All patients provided written informed consent according to the Declaration of Helsinki. The patients were treated with ANA after being approved by the Turkish Medicines and Medical Devices Agency.

Patient 1

A four-year-old Turkish boy of consanguineous (first cousins) parents was followed up by the pediatric neurology outpatient clinic. He was the second child of a healthy 26-year-old mother and a healthy 32-year-old father. He had a healthy sibling. He was delivered prematurely via Caesarean section at gestational age of 30 weeks. Birth head circumference was unknown. He was admitted to the neonatal intensive care unit due to prematurity and stayed for 46 days. Initially, he was referred to the pediatric neurology department due to an enlarged head circumference when he was aged 10 months, at which time he could not sit up without support. He was able to follow the one-step command with gestures and responded to the sound of his name. Upon neurological examination (10 months) there was no focal neurological finding. His head circumference was 51 cm (+3.5 standard deviation score [SDS]). Ultrasonography showed increased diffuse echogenicity in the periventricular white matter. Diffusely abnormal and mildly swollen white matter as well as subcortical cysts in the anterior temporal and frontoparietal regions were detected on brain MRI (Fig 1). Magnetic resonance spectroscopy showed a reduced *N*-acetyl aspartate/creatinine ratio. Sanger analysis of the *MLC1* gene was performed, and homozygous pathogenic variants IV58+5G>A [c.714+5G>A] were detected. Finally, he was diagnosed with MLC when he was aged 15 months. Electroencephalography was normal, and physical therapy was recommended.

When he was aged 26 months, he presented to the emergency department with a fever of 15 days and was hospitalized. No fever focus was detected upon physical examination. Microbiological tests were all negative. Propranolol was administered in consideration of central fever, but he was unresponsive. Papilledema was detected via fundus examination, and prednisolone 1 mg/kg/d was started. Subsequently, his fever and papilledema resolved; however, while tapering the steroid treatment he again developed fever. ANA, an anti-IL-1 drug, was initiated at 4 mg/kg/d when he was aged 31 months. At the time ANA treatment was initiated, he was not able to walk, but he could pull himself up to a standing position by holding on to furniture. He could not say anything meaningful,

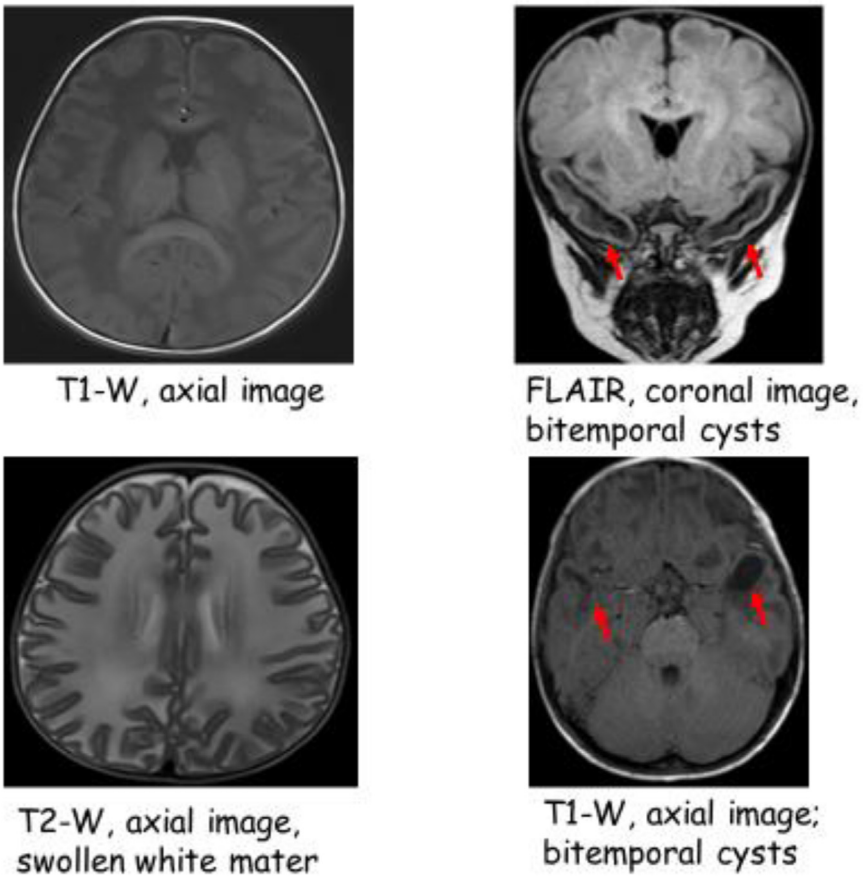

Figure 1. Cranial magnetic resonance imaging of Patient 1. FLAIR, fluid-attenuated inversion recovery; T1-W, T1-weighted; T2-W, T2-weighted.

except "daddy." His head circumference was 53 cm (+2.28 SDS). Volumetric MRI was performed by using the automated MRI brain volumetry system (vol2Brain). This system is an online system that provides automatically volumetric brain information.[5] The volumetric MRI showed that the cerebrum was 2019.51 cm^3, the white matter of the cerebrum was 1219.98 cm^3, the white matter of the cerebellum was 116.30 cm^3, the gray matter of the cerebrum was 585.69 cm^3, and the gray matter of the cerebellum was 97.54 cm^3. At month 7 of ANA treatment, his head circumference was still 53 cm. His follow-up volumetric MRI showed that the cerebrum was 1953.62 cm^3, the white matter of the cerebrum was 1134.97 cm^3, the white matter of the cerebellum was 100.44 cm^3, the gray matter of the cerebrum was 597.72 cm^3, and the gray matter of the cerebellum was 120.50 cm^3. Although there was an increase in cerebral and cerebellar gray matter volume, which could be compatible with age progression, a decrease of 3.26% in total brain volume was detected. This decrease was found to be associated with a significant decrease in both cerebral and cerebellar white matter volume, possibly related to the resolution of cerebral edema.

At month 7 of ANA treatment, his mother reported that he was able to stand up without support, he smiled in response to hearing her voice, and the quantity of eye contact, attention span, and his interest in the external environment increased. Following ANA treatment, the patient could take risks and his awareness of danger increased. The baseline and month 7 of ANA treatment volumetric MRI findings are summarized in Table 1. His psychometric test results at baseline and at month 7 of ANA treatment are shown in Table 2. No adverse effect was observed during the ANA treatment. At the time this manuscript was prepared the patient was aged 40 months.

Patient 2

A 14-year-old Turkish boy was followed-up at the pediatric neurology outpatient clinic. He was the first child of healthy nonconsanguineous parents, a 32-year-old father and a 26-year-old mother. He was delivered vaginally at term, but was admitted to the neonatal intensive care unit due to transient tachypnea and stayed there for 10 days. When he was aged seven months he presented to the pediatric neurology outpatient clinic due to developmental delay, macrocephaly, and seizure. At age seven years genetic analysis was performed, showing homozygous pathogenic variants (c.353C>g, pThr118Arg) in the *MLC1*, and he was diagnosed with MLC.

At the time MLC was diagnosed, he was not able to walk, speak, hold his head upright, or feed himself. Neurological examination showed no focal neurological finding. His head circumference was 56 cm (+2.75 SDS). Cranial MRI revealed increased diffuse echogenicity in white matter as well as subcortical cysts in the left frontal lobe (Fig 2). He was treated with carbamazepine, levetiracetam, baclofen, and risperidone. At age 14 years, he was referred to the pediatric neurology department due to seizures triggered by fever. At that time, he was still not able to walk, speak, hold his head upright, or feed himself. His head circumference was 62 cm (+4.46 SDS).

ANA 4 mg/kg/d was initiated when he was 13 years old. At the time of ANA treatment, volumetric MRI showed that the cerebrum was 1883,10 cm^3, the white matter of the cerebrum was 1219.71 cm^3, the white matter of the cerebellum was 105.32 cm^3, the gray matter of the cerebrum 482.58 cm^3, and the gray matter of the cerebellum was 75.78 cm^3. At month 18 of ANA treatment, his volumetric MRI showed that the cerebrum was 1854.05 cm^3, the white matter of the cerebrum was 1153.36 cm^3, the white matter of the cerebellum was 87.91 cm^3, the gray matter of the cerebrum was 515.88 cm^3, and the gray matter of the cerebellum was 96.90 cm^3. Although the total brain volume decreased by 1.54%, a decrease in cerebral and cerebellar white matter volume and an increase in cerebral and cerebellar gray matter volume were noted, similar to the other patient.

At month 18 of ANA treatment, his parents reported that the patient had experienced rapid developmental regression before the treatment, but the regression slowed after initiation of the treatment and in some cases stopped. Furthermore, whereas he had no control of his head before treatment, after ANA he could hold his head upright a little bit at the moment. He had no ability to walk before the treatment and was bedridden, but post-treatment he could sit up and change his position (for instance, move from a lying to a sitting position). In addition, post-treatment, his ability to understand verbal commands and expression of what he wants were improved. The baseline and eighteenth-month volumetric MRI findings are summarized in Table 1, and the psychometric test results at baseline and eighteenth month of ANA treatment are shown in Table 2. No adverse effect was observed during the ANA therapy. In addition, post-treatment he has not had any seizures. At the time this manuscript was prepared the patient was aged 14 years.

Discussion

Herein, we presented two patients with MLC who were treated with the anti-IL-1 drug, ANA. After treatment brain volumes in both patients decreased significantly, and their cognitive functions and social interactions improved. Astrocytes are the most abundant cell type in the CNS. These cells perform several important functions related to brain homeostasis, such as metabolic support for neurons, neurotransmitter uptake, buffering of extracellular K^+ ions, control of the blood-brain barrier, and blood flow.[6] Reactive astrocytes play a neuroprotective role in cases of CNS injury; however, when the feedback mechanisms do not process properly, their protective functions can evolve into malign functions. Mutations in astrocytic-specific proteins can cause myelin defects and

TABLE 1.
Volumetric Magnetic Resonance Findings of Patients

Part of the Brain	Patient 1			Patient 2		
	Pretreatment Volumes (cm^3)	Post-treatment Volumes (cm^3)	Volume Change	Pretreatment Volumes (cm^3)	Post-treatment Volumes (cm^3)	Volume Change
Brain (white matter + gray matter)	2019.51	1953.62	−3.26%	1883.10	1854.05	−1.54%
Cerebral white matter	1219.98	1134.97	−6.96%	1219.71	1153.36	−5.43%
Cerebellar white matter	116.30	100.44	−13.63%	105.32	87.91	−16.53%
Cerebral gray matter	585.69	597.72	+2.05%	482.58	515.88	+6.90%
Cerebellar gray matter	97.54	120.50	+23.53%	75.78	96.90	+27.87%

TABLE 2.
Evaluation of Cognitive Functions and Social Interactions of Patients

Cognitive Functions and Social Interactions	Patient 1			Patient 2		
	Pretreatment	Post-treatment	Changes	Pretreatment	Post-treatment	Changes
Pediatric evaluation of disability inventory						
Functional skills self-care scale	8	13	+5	22	19	−3
Functional skills mobility scale	39	44	+5	13	13	0
Functional skills social function scale	3	4	+1	22	30	+8
Dunn sensory profile test						
Sensory processing						
Auditory processing	15	21	+6	30	31	+1
Visual processing	27	24	−3	30	10	−20
Vestibular processing	41	42	+1	40	35	−5
Touch processing	56	57	+1	62	78	+16
Multisensory processing	15	16	+1	26	28	+2
Oral sensory processing	34	39	+4	54	50	−4
Modulation						
Sensory processing related to endurance and tone	38	39	+1	23	22	−1
Modulation related to body position and movement	35	35	0	40	25	−15
Modulation of movement affecting activity level	25	26	+1	24	16	−8
Modulation of sensory input affecting emotional responses	5	5	0	11	8	−3
Modulation of visual input affecting emotional responses and activity level	12	13	+1	15	4	−11
Behavior and emotional responses						
Emotional/social responses	54	53	−1	41	46	+5
Behavioral outcomes of sensory processing	16	16	0	13	23	+10
Items indicating thresholds for responses	15	15	0	13	15	+2

neurodegenerative disorders.[7] MLC1 is a membrane protein expressed by perivascular astrocytes. MLC1 acts as a regulator protein for decreasing volume after astrocyte osmotic swelling. Mutations in the *MLC1* result in leukodystrophy characterized by megalencephaly, brain edema, cysts, myelin vacuolation, and astrocytosis.

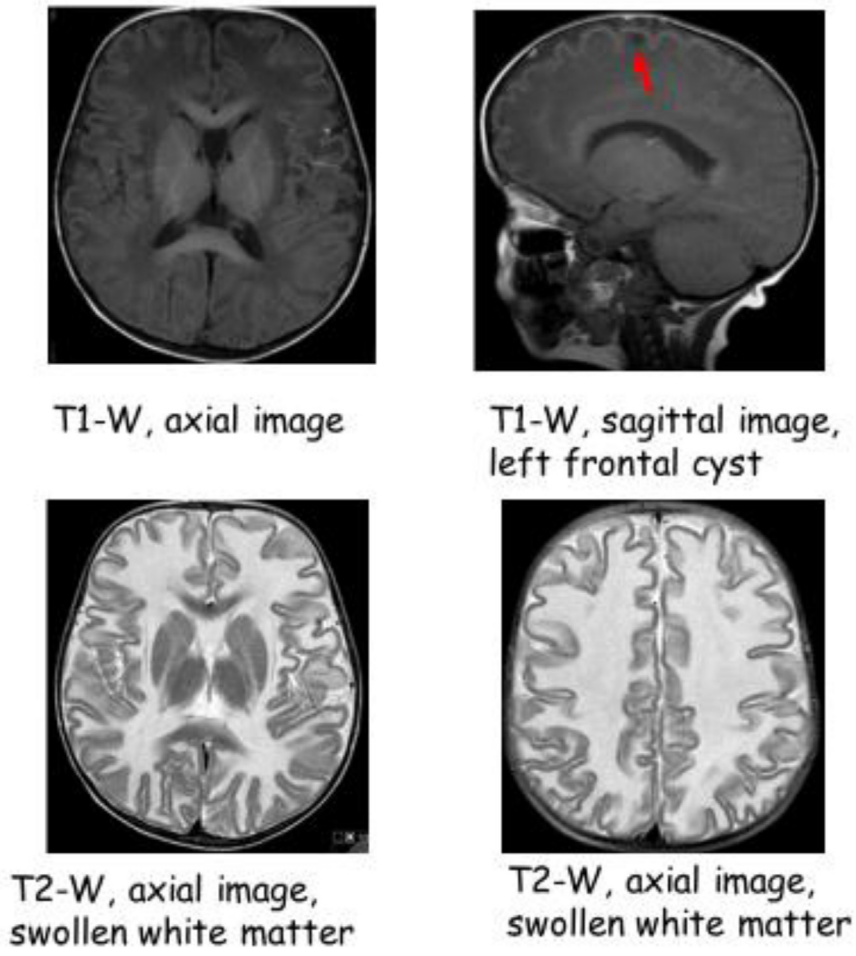

Figure 2. Cranial magnetic resonance imaging of Patient 2. T1-W, T1-weighted; T2-W, T2-weighted.

Earlier studies have shown that MLC-1 expression inhibits the activation of astrocyte signaling cascades in response to brain injury.[8] Animal studies may provide a model for understanding the pathophysiology of rare diseases.[3] Mice models show that MLC1 plays a role in astrocyte activation during neuroinflammation.[3] In mice, three different MLC1 knockout lines were produced and increased brain water volume was observed in MLC1 knockout mice; however, there were some regional differences in vacuolated areas between patients with MLC and MLC1 knockout mice.[9-11] Primarily, subcortical white matter is affected in patients with MLC, whereas vacuolation and brain edema predominate in the cerebellum of MLC1 knockout mice, which might contribute to differences in the expression of proteins in the brain.[9-11] In the present study resolution of brain edema was observed in both the cerebral and cerebellar parts of the brain white matter content.

To date, there is no curative treatment for MLC. Bosch et al.[3] reported that MLC1 knockout mice injected with adeno-associated virus coding for human MLC1 exhibited extremely decreased myelin vacuolation, suggesting that gene therapy might be a potential treatment.[3] Interestingly, Hamilton et al.[12] described the resolution of brain edema in patients with MLC2B, whereas white matter signal abnormalities persisted or increased in patients with MLC1 and patients with MLC2A. In the CNS physiological astrocyte swelling in response to neuronal activity and extracellular K^+ release can occur. MLC1 plays a central role in the regulation of astrocyte activation in both physiological responses and pathologic conditions.[11] Brignone et al.[4] investigated the role of MLC1 in astrocyte activation during neuroinflammation using human brain tissues and reported that MLC1 inhibits the activation of the IL-1β-induced inflammatory response, confirming the role of MLC1 in recovery processes and homeostasis following inflammation. IL-1β is among the primary inflammatory cytokines released by microglial cells in the presence of brain injury. Inhibition of IL-1β decreases inflammation and brain edema in animal models.[13,14] Based on the presented two patients, we think that dysfunctional MLC1 might result in impaired response to increased IL-1 and, hypothetically, that blocking IL-1 might slow the progression of brain edema and astrocytosis. The presented patients with MLC exhibited improvement in cognitive functions and social interactions and resolution of brain edema based on volumetric cranial MRI following ANA treatment. The volumetric MRI of patients showed evident resolution of brain edema in both cerebral and cerebellar white matter. There was a significant decrease in cerebral and cerebellar white matter volume and an increase in cerebral and cerebellar gray matter volume. Although there is a decrease in cerebral cerebellar white matter volume in the course of ANA treatment, the process of expected age-related volume increase in cortical gray matter volume is not affected. In the diagnosis of MLC, white matter changes in the cerebral white matter detected on T2- and T1-weighted images require differential diagnosis with some leukoencephalopathies; however, since subcortical cysts in the anterior temporal or frontoparietal region are specific for the diagnosis of MLC, radiological diagnosis is usually not difficult. The necessity of new imaging modalities in terms of diagnosis is limited; however, showing increased diffusion in white matter in diffusion sequences; decreased *N*-acetyl aspartate, creatinine, and choline levels and normal myoinositol levels in proton magnetic resonance spectroscopy; and increased apparent diffusion coefficient and decreased anisotropy in diffusion tensor imaging support the diagnosis. Compared with conventional MRI, volumetric MRI is a more sensitive automated method to examine structural alterations of the whole brain. Volumetric MRI allows segmenting and measuring of each part of the brain to assess neurodegeneration in earlier stages.[5] In the present study, we demonstrated the resolution of brain edema after treatment in two patients with MLC by using volumetric MRI. Furthermore, although there were improvements in cognitive functions, some functions had regressed under the treatment. We suggested that ANA treatment did not completely eliminate neurodegeneration, but could only slow it down. Patients with MLC may present with seizures, but the underlying mechanism of epilepsy in patients with MLC is yet to be clarified. Nonetheless, it is hypothesized that MLC proteins might act as ion channels. Patient 2 suffered from recurrent seizures triggered by fever, and following ANA treatment epileptic seizures no longer occurred. ANA is an IL-1 receptor antagonist. The therapeutic potential of ANA in patients with cerebral autoinflammatory response has been evaluated.[15-17] The most common side effect of anti-IL-1 treatment is injection site reaction, but mildly elevated transaminase or susceptibility to upper respiratory tract infections have also been reported.[18] In the presented patients no adverse effects were observed during ANA treatment.

There are some limitations of our study: levels of IL-1 or other cytokines were not measured before and after the treatment. Furthermore, evaluating volumetric MRI may be difficult at a young age due to natural head growth.

In conclusion, the presented patients indicate that ANA or other IL-1 antagonists can be used to suppress disease activity in patients with MLC, but potential interactions between other inflammatory pathways and MLC1 cannot be ignored; therefore, the present findings need to be confirmed by additional research. Unfortunately, suppressing inflammation alone is not a curative treatment for MLC, whereas IL-1 antagonists may slow the progression of the neurodegenerative process. However, this positive effect should be evaluated in new comparative longitudinal studies.

Acknowledgments

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