



Original Article

Corticosteroid Therapy for Duchenne Muscular Dystrophy: Improvement of Psychomotor Function

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ABSTRACT

BACKGROUND: Of the numerous clinical trials for Duchenne muscular dystrophy, only the corticosteroid prednisolone has shown potential for temporal improvement in motor ability. In this study, the effects of prednisolone on intellectual ability are examined in 29 cases of Duchenne muscular dystrophy because little information has been reported. And also, motor functions and cardiac functions were evaluated. **METHODS:** The treated group was administered prednisolone (0.75 mg/kg) orally on alternate days and the compared with the untreated control group. Gene mutations were investigated. The patients were examined for intelligence quotient adequate for age, brain natriuretic peptide, creatine kinase, and manual muscle testing before treatment and after the period 6 months to 2 years. **RESULTS:** Intelligence quotient scores of the treated increased to 6.5 ± 11.9 (mean \pm standard deviation) were compared with the controls 2.1 ± 4.9 ($P = 0.009$). Intelligence quotient scores of the patients with nonsense point mutations improved significantly (21.0 ± 7.9) more than those with deletion or duplication (1.9 ± 9.0 ; $P = 0.015$). Motor function, such as time to stand up, of those treated improved significantly and brain natriuretic peptide level was reduced to a normal level after treatment in 15 patients (73%). **CONCLUSIONS:** Our results demonstrate the effectiveness of prednisolone in improving intellectual impairment as well as in preserving motor function and brain natriuretic peptide levels. We presume that prednisolone has a read-through effect on the stop codons in the central nervous systems of Duchenne muscular dystrophy because intelligence quotient of point mutation case was improved significantly.

Keywords: Duchenne muscular dystrophy, prednisolone, cognition, motor function

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Introduction

Duchenne muscular dystrophy (DMD) is the most common type of childhood muscular dystrophy. It is characterized by progressive muscle weakness and deterioration of skeletal and cardiac muscle function. DMD is an X-linked recessive disorder, caused by mutations in the *DMD* gene in Xp21.1, leading to complete absence of the cytoskeletal

protein dystrophin in both skeletal and cardiac muscle cells.¹ Several treatments involving drugs with read-through effects have attracted major interest, such as the amino glycoside antibiotic gentamicin^{2,3} and PTC124⁴ as well as antisense morpholinos that cause exon skipping.⁵ These treatments are, however, being administered only as clinical research regimens at present and have not yet reached the stage of extensive clinical application. The only widely used clinically effective treatment for DMD patients is oral administration of prednisolone (PSL). This treatment was highly evaluated, as evidence level 1, by the US Neurology Academy and at the US Pediatric Neurology Meeting in 2005.⁶

PSL is believed to temporarily slow motor function decline and disease progression. In our daily examination of

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TABLE.
Comparison of two groups before PSL

	Treated Group (n = 20)*	Control Group (n = 9)*	t Test (P < 0.05)
Age at treatment onset (yr)	5.9 ± 0.2	6.1 ± 0.6	P = 0.7099
Time required to stand before PSL (seconds)	3.9 ± 0.3	4.6 ± 4.8	P = 0.5711
Gluteus maximus muscle strength before PSL	3.4 ± 0.1	3.4 ± 0.2	P = 0.2250
Iliopsoas muscle strength before PSL	3.4 ± 0.1	3.5 ± 0.3	P = 0.0944
CK (IU/L) before PSL	15,492 ± 1662	14,414 ± 1091	P = 0.3317

Abbreviations:
 CK = Creatine kinase
 PSL = Prednisolone
 SE = Standard error
 * Mean ± SE.

patients, we recognized that PSL administration improves responsiveness, verbal rapport, and motor functions in DMD patients. Thus, we hypothesized that PSL also has an ameliorating effect on central nervous system dysfunctions, including intellectual impairments.

In this study, we measured intelligence quotients (IQs) to evaluate intellectual abilities before and from 6 months to 2 years after starting PSL administration. We used the Stanford-Binet Intelligence Test (5th edition), which is the standardized version of the Stanford-Binet method applied in Japan for evaluating preschool children and the Wechsler Intelligence Scale for Children, 3rd edition, which is used in school-age children.

For motor function, we also measured the time required to stand up and lower limb strength using manual muscle testing. We measured serum creatine kinase (CK) titers to assess the state of skeletal muscles.

Furthermore, because 50% of DMD patients develop heart failure resulting from left cardiac dysfunction,⁷ plasma brain natriuretic peptide (BNP) levels were also measured to evaluate cardiac muscle function. BNP served as a marker of cardiac function in the treated group. BNP is known to be an adequately sensitive marker for detection of stage 1 heart failure. Furthermore, Sakurai et al reported correlations between plasma BNP level and indices of cardiac function.⁸

Materials and Methods

Identification of genetic mutations

Mutations were investigated in all patients to detect deletion or duplication of genes by multiplex polymerase chain reaction or multiplex ligation-dependent probe amplification using a Holland P034/P035 DMD kit (FALCO Biosystems). In patients in whom neither method revealed any abnormalities, point mutation analyses employing complementary DNA direct sequencing were performed with messenger RNA extracted from peripheral blood lymphocytes or biopsied muscle tissues.

Clinical analyses before and after PSL administration

Twenty-nine DMD patients were divided into a treated group and a control group. The 21 patients in the treated group (mean age: 5.9 years) were outpatients between 1994 and 2010 at the Department of Pediatrics, or the Institute of Medical Genetics of Tokyo Women's Medical University. PSL (0.75 mg/kg) was orally administered on alternate days.^{9–11} The other eight patients, all with identified gene mutations, comprised the control group (mean age: 6.1 years). The other eight

patients had been followed at the Department of Pediatrics between 1980 and 1990 and had detailed medical records available (Table).

Investigation of IQ scores

IQ scores were examined before PSL administration and also 6 months to 2 years after starting PSL. IQ scores are numerical values that express the results of an intelligence test. A score of 80 or above is considered to be within normal range, whereas a score between 70 and 79 is borderline, 50 to 69 indicates mild intellectual impairment, 35 to 49 indicates moderate intellectual impairment, and 35 or below is classified as severe intellectual impairment.¹² Subjects were divided into three groups based on the degree of IQ score changes, namely, an increase group, in which IQ scores increased by 10 or more points; an unchanged group, in which IQ score changes were within 0 to 10 points; and a decrease group, in which IQ scores dropped by 10 or more points after PSL administration.

The IQ testing method was selected according to the age and developmental stage of each subject. The IQ testing methods differ before and after school age. In preschool children, IQ might be measured by WPPSI (Wechsler Preschool and Primary Scale of Intelligence). In Japan, however, the WPPSI-IV is not as yet a standard test, whereas the WPPSI-III was in use for 30 years, although it would not now be considered appropriate. Therefore, a standardized version of the Stanford-Binet method has been adopted for preschool children. For school-age children, IQ should be measured employing the Wechsler Intelligence Scale for Children, 3rd edition, which is used to evaluate and measure both performance and verbal IQ. We adopted the Japanese version of Wechsler Intelligence Scale for Children, 3rd edition, for school-age children in this study.

Measurement of motor functions and CK and BNP levels before and after PSL administration

The effects of PSL were observed by measuring motor functions, including the time required to stand up and muscle strength by manual muscle testing, to compare the treated and control groups. Furthermore, because 50% of DMD patients develop heart failure resulting from left cardiac dysfunction, BNP levels were measured in the treated group to allow comparison of cardiac functions among genetic mutation types.

Time required to stand up. The time required to stand up from a supine to a standing position was measured in 18 patients in the treated group and eight in the control group.

Muscle strength. Muscle strength was measured in 16 of the treated patients and in the eight control group patients who had periodically undergone manual muscle testing. Because DMD is characterized by proximal muscle weakness, the strength of the gluteus maximus and iliopsoas muscles were measured under conditions of both supine hip flexion and prone hip extension. The strength of the gluteus maximus and iliopsoas muscles were also investigated. All physical examinations, including the manual muscle testing, were conducted by the same doctor.

CK levels. Levels of CK, the skeletal muscle enzyme, were measured in 15 of the treated patients and in the eight control patients, with values of

200 IU/L or less being considered normal. CK titers were studied at regular visits to our institute. The activity levels on the day of measurement and the previous day were not taken into consideration.

BNP levels. BNP levels were measured in 15 of the 21 treated patients, with 18 pg/mL or less being considered normal. BNP is known to be an adequately sensitive marker to detect heart failure at stage 1, when clinical symptoms are not yet apparent. Sakurai et al (2003) reported the plasma BNP level to correlate with indices of cardiac function. No patients in the treated group were taking parasympathetic agents, adenosine receptor modulating drugs, or angiotensin-converting enzyme inhibitors, all of which can affect cardiac function. BNP levels and gene mutation types were compared.

This study was approved by the Ethics Committee of Tokyo Women's Medical University (no. 2116).

Results

Identification of genetic mutations

Of the 29 study participants undergoing genetic analysis, nonsense point mutations were identified in five (control group 0; treated group 5), exon deletions in 22 (control

group 8; treated group 14), and exon duplications in 2 (control group 0; treated group 2).

Clinical and biochemical analyses before and after PSL administration

Investigation of IQ scores

The IQ level had increased significantly in the treated group 6 months to 2 years after starting treatment, as compared with the control group. IQ scores of the treated group were thus increased by 6.5 ± 11.9 points (mean \pm SD) as compared with those of the controls (2.1 ± 4.9) ($P = 0.009$) (Fig 1A, B). Differences among the three genetic mutation types were also compared and the IQ scores of patients with nonsense point mutations were increased 21.0 ± 7.9 points, showing a greater improvement than those with deletion or duplication mutations (1.9 ± 9.0) ($P = 0.015$) (Fig 1C, D).

Measurements of motor functions and CK and BNP levels

Time required to stand up. The time required to stand up in the control group was 5.7 ± 1.3 seconds (mean \pm SD), showing a

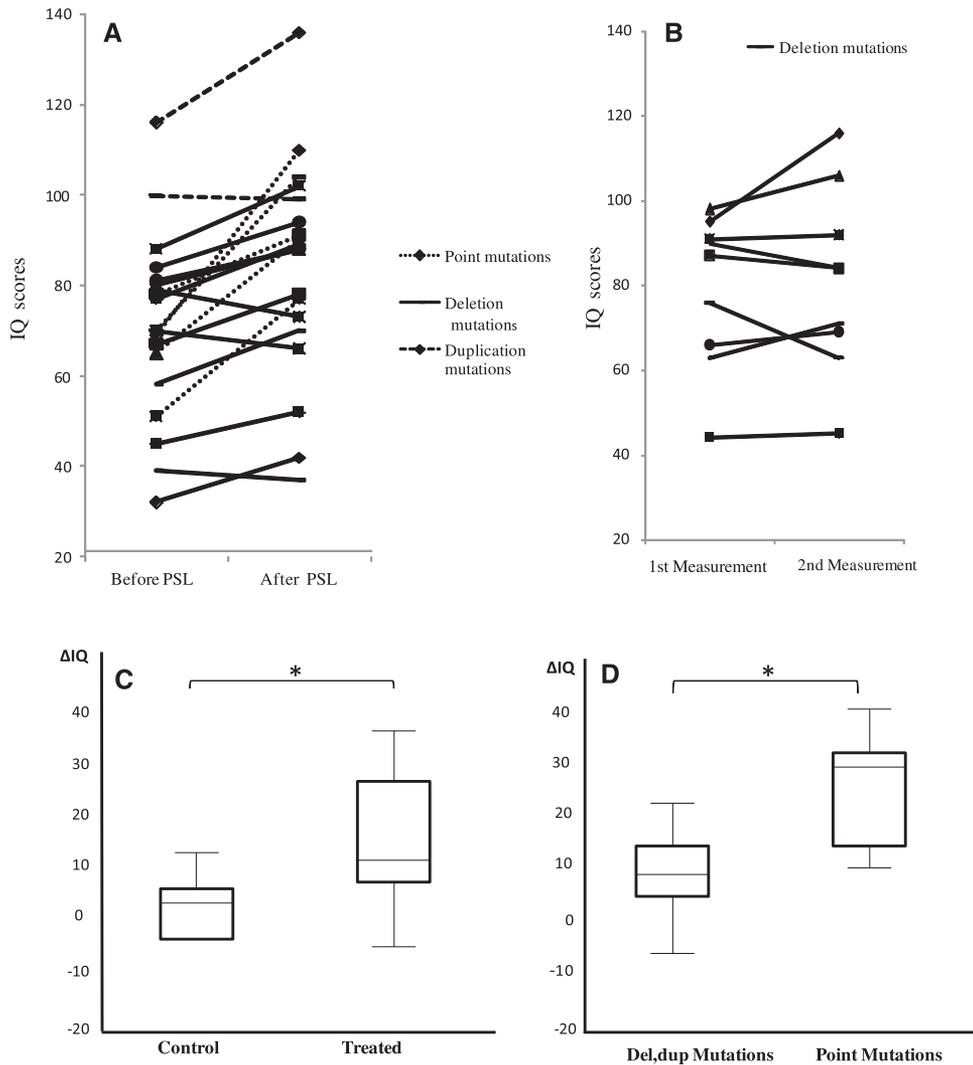


FIGURE 1. Investigation of IQ scores after PSL. (A) IQ scores of treated group; (B) IQ score of control group; (C) Δ IQ scores compared between the above two groups ($P=0.009$); (D) Δ IQ scores compared between nonsense point mutation and a group of deletion + duplication ($P=0.0015$).

marked increase over time, whereas that of the treated group was 3.3 ± 0.2 seconds and was thus unchanged 6 months to 2 years after the start of PSL treatment ($P = 0.031$) (Fig 2A).

Muscle strength. The strengths of the gluteus maximus and iliopsoas muscles were investigated for 6 months to 2 years. Gluteus maximus muscle strength differed significantly between the two groups after 2 years. Iliopsoas muscle strength was at 3.5 ± 0.9 (mean \pm SD) in the treated group, whereas it decreased to 3.1 ± 0.2 in the control group ($P = 0.048$) (Fig 2B). However, the difference between the two groups did not reach statistical significance ($P = 0.222$).

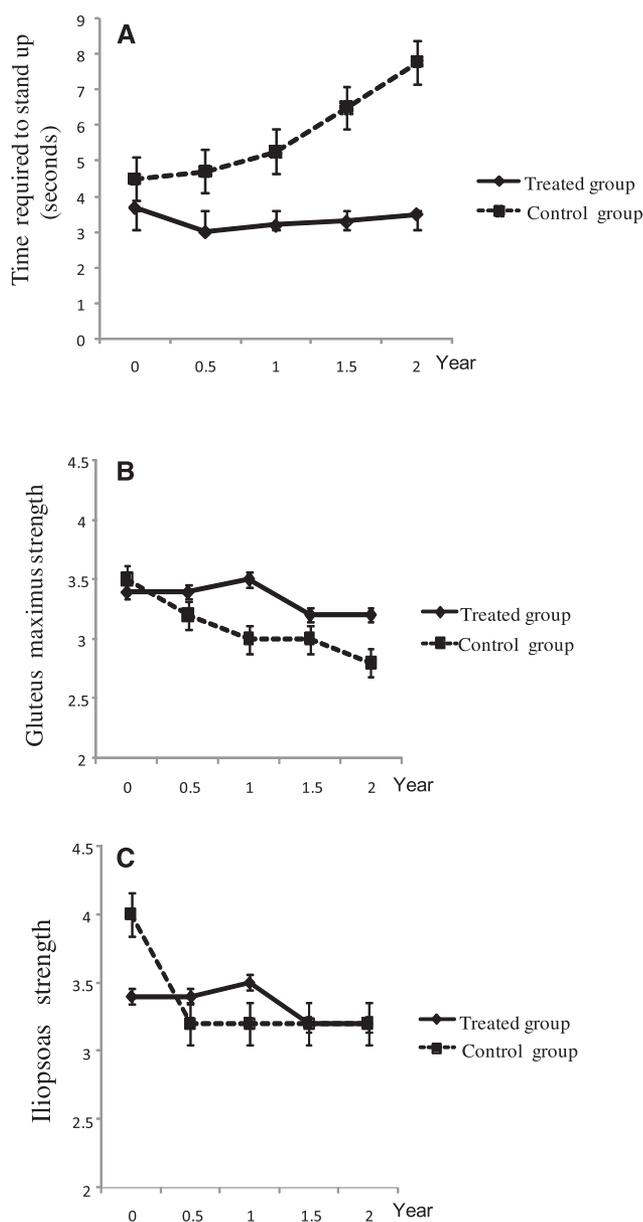


FIGURE 2. Evaluation of the muscular power after PSL. (A) The times required to stand up between treated and control groups ($P=0.031$); (B) The strength of the Gluteus maximus muscle by MMT between 2 groups ($P=0.048$); (C) Iliopsoas muscle strength by MMT between two groups ($P=0.222$).

CK levels. The patients had high CK levels, which is common for pediatric DMD patients at the onset of treatment. However, these levels did not change significantly over the course of treatment and there was no significant difference between the two groups at the end of follow-up ($P = 0.505$) (data not shown).

BNP levels. After PSL administration, the BNP levels of 15 patients (73%) in the treated group were normal. Of those 15 patients, four had nonsense point mutations, nine had deletion mutations, and two had duplication mutations. BNP was significantly lower at 5.1 ± 4.0 (mean \pm SD) in patients with point mutations 1 year after the start of PSL administration than the level of 7.7 ± 5.5 in those with deletion and duplication type mutations ($P = 0.034$) (Fig 3).

Discussion

DMD patients are generally diagnosed at 2-3 years of age. There is a steady decline in motor function after age 6. By age 10, braces may be required for walking, and by age 12, patients are confined to a wheelchair. Most are bedridden by approximately age 15. A few individuals with DMD who live beyond their 30s require artificial ventilation because of diaphragm muscle failure. Intellectual abilities vary widely among DMD patients. Although some have normal intellectual abilities, others exhibit intellectual impairments. A few patients do not even attain meaningful words. Others also have developmental disabilities such as autistic spectrum disorders or attention deficit hyperactivity disorders. Many studies on the IQ scores of DMD patients have already been published.¹² The mean IQ score among these reports was reported as 82. Until the 1950s, the intellectual disability observed in some DMD patients was attributed to secondary effects of motor disability and muscle weakness, namely physical and social barriers in addition to the lack of educational opportunities.¹³ It was subsequently recognized that intellectual impairment is present before the appearance of the symptoms of muscle weakness and atrophy. Intellectual impairment was found to be nonprogressive and there were no correlations among IQ scores, age, and disease progression.¹⁴⁻¹⁶ There is, at present, no evidence supporting the concept that intellectual impairment in DMD patients is a secondary effect of muscle weakness. Intellectual impairment is now considered to be caused by genetically determined dysfunctions impacting the central nervous system.

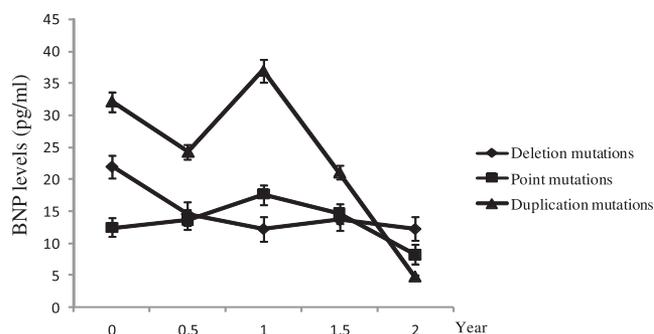


FIGURE 3. Transition of BNP levels after PSL treatment among 3 gene mutation groups; the lowest in nonsense point mutation.

When PSL treatment for DMD patients was started, the daily dosage was high at 2 mg/kg/day. As a result, various adverse reactions were observed, such as cushingoid facial features, weight gain, and behavioral abnormalities. Thereafter, the establishment of appropriate doses was investigated by comparing side effects at PSL doses of 1.5, 0.75, and 0.3 mg/kg/day administered daily for 6 months by a consortium on muscular dystrophy.⁹ In the present study, the selected PSL dose was administered as an alternate-day medication regimen of 0.75 mg/kg/day.¹⁷ Converted into a daily dose, this would be within a daily medication range of 0.12–0.5 mg/kg/day, which is a commonly prescribed amount. Efficacy not inferior to that of a daily medication regimen is provided, with the major advantage of avoiding adverse reactions.

Evaluation of intellectual ability before and after PSL administration

Along with physical problems, mental problems have been recognized in association with DMD. It has been estimated that approximately one third of DMD patients suffer from mental retardation or other forms of intellectual impairment. The IQs of DMD patients are reportedly in the range of 68–91, with the average being in the mid-80s. Characteristically, verbal IQ has been somewhat better than performance IQ.¹⁸

Intellectual impairment does not appear to progress as motor function deteriorates.^{19,20} In this study, the mean IQ score of our 29 patients was 85, which is consistent with previous reports. Also, IQ scores generally remain constant. Although slight fluctuations may occur because of environmental factors, changes exceeding 10 points are considered rare.²¹ This was also supported by the observations of our control group in which IQ scores did not change. However, when we started PSL treatment, improvements in verbal rapport and reactions were clearly recognized clinically in several patients, leading us to hypothesize that PSL had exerted a beneficial influence on intelligence.

In this study, the maximum IQ score increase was 23 points in a treated patient, which is an extraordinary improvement (Fig 1A). IQ scores were increased as compared with those of the control group ($P = 0.009$) (Fig 1C).

No previous studies, to our knowledge, have examined the effects of PSL on intellectual ability in pediatric patients. For adult patients, effects of PSL on the central nervous system are suggested to represent modulations of neurological symptoms such as mood disorders including depression and mania, psychiatric disorders such as delirium and hallucinations, and cognitive or memory impairments. There are several possible mechanisms by which PSL might exert such effects on neurological symptoms. One possibility is the strong affinity of PSL for receptors, particularly those of the limbic system including the hippocampus, all because PSL may affect the activities of regional neurotransmitters. Other possible mechanisms include involvement of central synapses in prolonged neurotransmission latency periods associated with changes in blood-brain barrier permeability and impairments of cerebral metabolic enzymes. Any or all of these factors might be mechanisms underlying neurological symptom onset.²² However, none of these neurological symptoms

was found in our DMD patients. Instead, improvements of verbal rapport, reactions, and IQ scores were observed. Thus, we hypothesized that PSL acts differentially on the central nervous system in DMD patients.

Symptoms of DMD are accounted for by lack of dystrophin protein. Dystrophin is mostly expressed in muscles, with the next highest level of expression being in the brain.²³ Intracranial dystrophin is known as cerebral dystrophin and exists in the postsynaptic membrane, which is a neurotransmitter circuit.²⁴

If PSL accelerates muscular dystrophin production, cerebral dystrophin would also be increased, thereby ameliorating intellectual impairment. Cerebral dystrophin comprises various isoforms produced from one gene, and the isoforms exist in the postsynaptic membrane, which is a neurotransmitter circuit. Because various isoforms of dystrophin exist in neurons in the central nervous system, it is possible that mutation of the DMD gene would result in dysfunction of neurons, thereby influencing not only intellectual levels but also determining specific neuropsychological profiles. However, there is still a great deal of uncertainty regarding the roles played by these various isoforms.²⁵

An experiment with mdx mice demonstrated that cerebral dystrophin was extensively distributed throughout the hippocampus, which is chiefly related to memory, the cerebellum, and the olfactory bulbs, governing sensation and motor function, and the thalamus, which has important roles in activity and consciousness levels.²⁶

It has also been reported that abnormalities of cerebral dystrophin isoform formation can cause central nervous system structural anomalies, dendritic cell defects, and decreased numbers of neurons.²⁷ This suggests that cerebral dystrophin anomalies resulting from abnormal brain isoform formation might be the primary cause of intellectual impairment. If PSL promotes the formation of dystrophin expressed in muscle cells, it must also activate production of cerebral dystrophin in the central nervous system, leading to shorter synaptic neurotransmission latencies and thereby to amelioration of the intellectual impairments.

Another interesting effect of PSL on intellectual ability, demonstrated in the present study, was a significant increase in the IQ scores of patients with nonsense point mutations as compared with those with deletion or duplication mutations (Fig 1D). Premature stop codons caused by nonsense point mutations lead to protein deficiencies and, in many cases, to loss-of-function effects. Read through refers to the treatment effect on patients with specific protein deficiencies resulting from nonsense point mutations. When certain chemical compounds, such as amino glycoside antibiotics, are administered to these patients, they act on ribosomes to read through the premature stop codon, and normal wild-type proteins are thus synthesized, leading to cure of the disease. Because significant increases in IQ scores were observed in patients with nonsense point mutations in this study, we speculated that PSL might exert read-through effects on stop codons and thereby lead to restoration of dystrophin expression in the brain. Our results also raise the possibility that other drugs with read-through effects may improve intelligence in DMD patients.

Evaluation of motor functions and CK and BNP levels with PSL administration

Treatment for DMD patients has been attempted with various medication regimens because no definitive therapy exists. At present, PSL is the only medication known to be effective.^{28,29} PSL improves muscle strength and motor function as well as delaying the progression of symptoms in DMD patients, although only temporarily. As shown in Fig 2A, the time required to stand up gradually increased in the control group over the course of 2 years. In contrast, the treated group showed no increase in the time required to stand up. We also measured lower limb muscle strength with manual muscle testing because muscle weakness in DMD patients is characterized by reduced proximal muscle strength. Muscle strength was partly maintained in the treated group, while gradual muscle atrophy occurred in the control group (Fig 2B,C).

There are related reports on research that include a previous preliminary study conducted in our laboratory. In that study, PSL was found to increase dystrophin expression in healthy muscles and also in the muscles of DMD patients. An experiment with mdx mice also reportedly demonstrated that PSL administration increased the productions of skeletal muscle dystrophin, spectrin, desmin, and actin proteins.³⁰

Although studies have elucidated a relationship between PSL and dystrophin production, more substantial clinical trials are required. In this study, the time required to stand up and lower limb muscle strength both showed improvement and were subsequently maintained in the treated group as compared with the control group. This suggests that PSL administration either stops, or perhaps even partially reverses, the degeneration skeletal muscle.

CK levels did not differ significantly between the treated and control groups (data not shown). Generally, rising CK titers are observed in relation to the amount of muscle contraction and fluctuate based on how much DMD patients move.³¹ The mean CK level of 6-year-old male DMD patients without PSL treatment was reported to be $10,611.1 \pm 4236.9$ IU/L ($n = 19$). From approximately 10 years of age, CK levels slowly decline but do not recover to normal levels.³² In the present study, CK levels in patients remained high from the beginning of the treatment period; none of our patients reached normal levels during the follow-up period. Reevaluation under standardized conditions is necessary to examine the relationship between PSL and CK levels.

In this study, although BNP levels were high before PSL administration, BNP had normalized in 73% of patients 1 year after starting PSL treatment (Fig 3). Evaluation at 2 years after starting PSL administration showed that all 15 patients had normal levels. This indicates PSL to be useful for improving BNP levels, again confirming its effectiveness in the treatment of DMD.

Conclusion

PSL administration significantly ameliorated intellectual impairments in DMD patients, in addition to improving motor function. We observed that patients with nonsense point mutations had more significant increases in IQ scores than those with other gene mutation types such as deletion

or duplication, which suggested that PSL might exert a read-through action on stop codons. DMD has been treated with various medications, including PSL, and the results of this study confirm the usefulness of this pharmacologic treatment not only for preserving motor ability, but also for improving intellectual ability in afflicted patients. This is the first study to clinically demonstrate significant intellectual improvement in DMD patients receiving PSL treatment. We anticipate that these results will contribute to future management of DMD patients. Going forward, more detailed investigations are needed. The pharmacologic effects of PSL on cerebral dystrophin as well as the stop codon read-through mechanism require verification in experiments using mdx mice.

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