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원 저

제2형 뮤코다당증의 임상적 스펙트럼과 효소대치요법의 단기간 효과

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Clinical Spectrum and Short-term Effects of Enzyme Replacement Therapy for Mucopolysaccharidosis Type II

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Purpose: We aimed to delineate clinical spectrum and short-term effects after enzyme replacement therapy (ERT) for 5 mucopolysaccharidosis type II (MPS II).

Methods: Five patients were diagnosed with MPS II by clinical findings, enzyme activity, and genetic testing. Idursulfase was administered by intravenous infusion at a dose of 0.5 mg/kg every week. Observational chart analysis of patients, who underwent systematic investigations more than 12 months after initiation of ERT was done retrospectively.

Results: Three patients were classified as having the attenuated type, and 2 patients were classified as having the severe type. The median age at the diagnosis was 9.6 years (range 3.4-26 years). Four different mutations in 5 Korean patients (4 families) with MPS II were identified, among which two were novel mutations (1 small insertion mutation: p.Thr409Hisfs*22, and 1 missense mutation: p.Gly134Glu). Two severe type sibling patients with the same mutation had different clinical manifestation. Urinary glycosaminoglycan excretion decreased within the twelve months of ERT (P=0.043). Liver and spleen volumes showed reductions that were maintained in all patients (P=0.043 and P=0.043, respectively). Improvements were also noted in left ventricular mass index (P=0.042), shoulder flexion (P=0.043), shoulder abduction (P=0.039), knee flexion (P=0.043), elbow flexion (P=0.042), and respiratory distress index (P=0.041).

Conclusion: This study demonstrates that Korean patients with MPS II are clinically heterogeneous and indicates that idursulfase is relatively effective in several clinical parameters including heart size and respiratory distress index without infusion-related reactions in patients with MPS II.

Key words: Mucopolysaccharidosis type II, Clinical spectrum, Idursulfase

Introduction

Mucopolysaccharidosis type II (MPS II or Hunter syndrome) is an X-linked recessive disease caused

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by a deficiency of the lysosomal enzyme iduronate-2-sulfatase (*IDS*), which catalyses the degradations of the glycosaminoglycan (GAG) dermatan sulfate and heparan sulphate[1,2]. The resulting lysosomal accumulation of upstream metabolites affects a variety of organ systems, including the visceral organs, skeleton, connective tissue, and the central nervous system[3,4]. MPS II occurs worldwide with an incidence of about 1 per 162,000 births[5]. Its common clinical manifestations are; coarse facial features, upper airway obstruction, cardiac valve regurgitation, restrictive lung disease, hepatosplenomegaly, hernias, joint contractures, and reduced quality of life[6]. More than 400 different genotypic variations have been documented in the *IDS* gene, which is located at Xq28.16. Variation in mutations type of *IDS* gene results in differences in symptoms within patients [7]. Recombinant human *IDS* (Idursulfase, Elaprase®) was approved for the treatment of MPS II by the Korea Ministry of Food and Drug Safety in 2009. Our goals in this study were to investigate the clinical manifestation and short-term clinical efficacy and safety of ERT for 5 patients with Korean patients with MPS II in a single center.

Patients and Methods

1. Patients

Five male patients from four unrelated Korean families were diagnosed to have MPS II at the Pusan National University Children's Hospital. The diagnosis of MPS II was confirmed by reduced or undetectable *IDS* enzyme activity in leukocyte and genetic testing. Mutation analysis and clinical review of these patients were approved by the institutional review board at Yangan Pusan National Hospital (IRB Number: 05-2013-073). Five patients were treated with idursulfase (Elaprase®), at a dosage of 0.5 mg/kg weekly, for more than 12 months. Comparison of efficacy changes between before and after 12 months of treatment with idursulfase was performed, including urinary GAG, liver/spleen volume, 6-Min Walk Test, left ventricular mass index (LVMI), Forced vital capacity, respiratory distress index (RDI), passive joint range of motion, and growth velocity. The change from baseline was analyzed with a Wilcoxon signed-rank test. All statistical calculations were performed with SPSS version 21.0.

2. Molecular genetic analysis

Genomic DNA was isolated from peripheral blood leukocytes of each patient using the PUREGENE DNA isolation kit (Gentra, Minneapolis, MN, USA). All 9 coding exons and exon-intron boundaries of the *IDS* gene were amplified by PCR on a thermal cycle (Applied Biosystems, Foster city, CA, USA) with primer pairs by the authors. Direct sequencing was performed with the BigDye Terminator V3.0 Cycle Sequencing Ready Reaction kit (Applied Biosystems) on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems). The sequences were analyzed using the with SeqScape v.2.5 (Applied Biosystems) and were compared to the reference sequence. Sequence variation was described according to the recommendations of the Human Genome Variation Society (www.hgvs.org/mutnomen) using a reference sequence (NM_000202.5). The pathogenic probability for each sequence variation of novel mutations in the *IDS* gene was predicted automatically by software MutationTaster (http://www. mutationtaster.org/).

Results

1. Clinical and molecular characteristics

We identified five Korean patients (3 attenuated type and 2 severe type) with MPS II. The median patient age at the diagnosis was 9.6 years (range 3.4-26 years). The median age of the patient at assessment was 9 years (range 3-25 years). Biochemical analysis results, genotype, and phenotype characterization of all patients were summarized in Table 1. Patient 1-3 were diagnosed with attenuated type MPS II without neurologic involvement. Patient 1 presented with stunted growth at 9 years of age. He had episodes of recurrent acute otitis media after 3 years. At 4 years of age he

Table 1. Summary of Clinical and Genotypic Characteristics of 5 Korean Patients with MPS II

No. of patients	1	2	3	4	5
Height (percentile)	10-25	<3	<3	75-90	<3
Body weight (percentile)	50-75	25	3-5	>97	<3
Age at diagnosis (years)	9.6	26	12	3.4	4.9
Age at first symptom (years)	4	6	5	2	2
Phenotype	attenuated	attenuated	attenuated	severe	severe
Coarse facial features	present	present	present	present	present
Hepatosplenomegaly	present	present	present	present	present
Dysostosis multiplex	present	present	present	present	present
Developmental delay/ mental retardation	absent	absent	absent	present	present
SNHL	both	both	both	both	both
Valve disease	MV/AV thickeness	severe MV stenosis MV regurgitation	MV thickeness MV prolapse	MV thickeness MV prolapse	MV thickeness
EMG/NCV	CTS	CTS	CTS	CTS	CTS
Brain MRI findings	normal	diffuse brain atrophy and multiple small low densities in periventricular deep white matter	normal	hydrocephalus	Chiari type I malformation
Operation	adenoid-tonsillectomy	repair of uh, replacement of MV	repair of ih and uh	repair of uh	meningomyelocele repair
Inheritance	familial	familial	familial	familial	familial
Phenotype	attenuated	attenuated	attenuated	severe	severe
Urine GAG (Ref: <36 mcg/mL)	220.06	37.00	943.00	535.00	927.00
IDS activity (Ref: 18-57 nmol/4hr/mg protein)	1.03	0.30	0.40	0.10	0.03
Nucleotide change	c.1224_1225insC	c.401G>A	c.187A>G	rearrangement	rearrangement
Amino acid change	p.Thr409Hisfs*22	p.Gly134Glu	p.Asn63Asp	lacking exon 4,5,6,7	lacking exon 4,5,6,7
Location	Exon 9	Exon 3	Exon 3	Intron 3,7	Intron 3,7
Novelty	novel	novel	known	known	known
Start of ERT (years)	9.7	26.3	12.2	3.5	5
Duration of ERT (months)	26	18	21	12	12

Abbreviations: GAG, glycosaminoglycan; IDS, iduronate-2-sulfatase; ERT, Enzyme replacement therapy; m, months; SNHL, Sensorineural hearing loss; Mitral valve, MV; Aortic valve, AV; Carpal tunnel syndrome, CTS; PPN, peripheral neuropathy; inguinal hernia, ih; umbilical hernia, uh.

underwent adenoid-tonsillectomy. Coarse facial features, including macrocephaly were noted. His height was between the 10th and 25th percentiles. He had hepatosplenomegaly and dysostosis multiplex. In addition, he had high tone sensorineural hearing loss, reduced pulmonary function, and carpal tunnel syndrome (CTS). He showed high intelligence (IQ score 140). He showed elevated urinary GAG level (220.06 mcg/mL; reference range (RR), <36 mcg/mL) and decreased *IDS* enzyme activity (1.03 nmol/4hr/mg protein; RR, 18-57 nmol/4hr/mg protein) in leukocyte. Mutation analysis revealed a 1-bp small insertion mutation, c.1224_1225insC (p.Thr409Hisfs*22) in exon 9, which caused frameshifts starting from codon 409 with a premature stop codon. This hemizygous mutation was derived from his mother (p.Thr409 Hisfs*22) and has not been reported previously. Patient 2 complained of dyspnea due to severe mitral valve stenosis and pulmonary hypertension at 26 years of age. At 2 years of age he underwent umbilical hernia operation. He had a profound short stature, hepatosplenomegaly and dysostosis multiplex. He had undergone mitral valve replacement. He showed normal intelligence (IQ score 115). He had a slightly elevated urinary GAG level (37.0 mcg/mL; RR, <36 mcg/mL) and showed decreased *IDS* enzyme activity (0.3 nmol/4hr/mg protein; RR, 18-57 nmol/4hr/mg protein) in leukocyte. DNA studies revealed a missense mutation, c.401G>A (p.Gly134Glu) in exon3, which was novel mutation and derived from his mother (p. Gly134Glu). This novel variant identified in the patient, is expected to be a mutation by in silico analysis. Patient 3 complained of a profound short stature. He had coarse facial features, CTS, and joint contracture; and had previously undergone surgery for an inguinal and umbilical hernia at 6 years of age. He showed high intelligence (IQ score 135). He had an elevated urinary GAG level (120 mcg/mL; RR, <36 mcg/mL) and diminished *IDS* enzyme activity (0.4 nmol/4hr/mg protein; RR, 18-57 nmol/4hr/mg protein) in leukocyte. A known missense mutation, c.187A>G (p.Asn63 Asp) in exon 3, was detected in this patient. Patients 4 and 5 were siblings and diagnosed with severe type MPS II with neurologic involvement. Patient 4 complained of intellectual disability (second grade level) at 3 years of age. He had hepatosplenomegaly, dysostosis multiplex and Mongolian spots. He had undergone an umbilical hernia operation at two years of age, and had markedly elevated urinary GAG level (535.0 mcg/mL; RR, <36 mcg/mL) and reduced *IDS* enzyme activity (0.1 nmol/4hr/mg protein; RR, 18-57 nmol/4hr/mg protein) in leukocyte. DNA studies revealed an *IDS-IDS2* recombination mutation. Patient 5 complained of intellectual disability (first grade level) at 3 years of age. He had a profound short stature, hepatosplenomegaly, and dysostosis multiplex. He underwent a meningomyelocele operation at age three years, and showed a highly elevated urinary GAG level (927.0 mcg/mL; RR, <36 mcg/mL) and reduced *IDS* enzyme activity (0.03 nmol/4hr/mg protein; RR, 18-57 nmol/4hr/mg protein). He had the same mutation as his sibling. He showed severe cognitive impairment (IQ score 40) at the age of 5 year old.

2. Efficacy and safety of enzyme replacement therapy

Five male patients, ages 3-26 years, received weekly intravenous infusions of 0.5 mg/kg idursulfase for 12 months. Most patients, including an adult with MPS II, showed several clinical improvements during the study (Table 2). Urinary GAG excretion decreased rapidly within the twelve

months of treatment (P=0.043). Liver and spleen volumes also showed reductions that were maintained in all patients by 12 months (P=0.043 and P=0.043, respectively). Improvements were also noted in LVMI (P=0.042), shoulder flexion (degrees) (P=0.043), shoulder abduction (P=0.039), knee flexion (degrees) (P=0.043), elbow flexion (degrees) (P=0.042), and RDI (P=0.041). However, two severe type patients with MPS II did not show improvement of neurological deficit and the neurological imaging findings did not show marked improvement in attenuated and severe type patients with MPS II. No infusion-related reactions occurred in all patients.

Discussion

This study was performed to investigate the clinical spectrum and short-term clinical efficacy and safety of ERT in Korean patients with MPS II. Clinically, MPS II should be regarded as a continuum between the two extreme forms of the disease (severe and attenuated)[8]. In the present study, three patients were classified as having the attenuated type, and 2 patients were classified as having the severe type. However, two sibling patients (patient 4 and 5) with the same mutation had different clinical manifestation: Patient 5 had a profound short stature and underwent a meningomyelocele surgery. However, patient 4 had a normal stature and severe Mongolian spots on whole body and underwent an umbilical hernia. This result suggested the clinical heterogeneity of MPS II. Although the phenotype of MPS II depends on the mutation types and deletions at the *IDS* gene[8-10], no strict relationship between genotype and clinical phenotype has been established[11]. In MPS II patients, more than 400 different genotypic variations have been documented in the *IDS* gene, which is approximately 24 kb in length with 9 exons[7]. Furthermore, it has been estimated that 55% to 57% of these are missense variations, 21% are nonsense variations, 14% to 20% are small deletions (20 bp), and 4% to 10% are major structural alterations such as large deletions (<20 bp) and rearrangements[10-12]. In this study, we reported 4 mutations in 5 Korean patients (4 families) with MPS II, that is, 2 missense

Table 2. Comparison of Efficacy Changes between before and after 12 Months of Treatment with Idursulfase

	Number	Baseline	12 months	Change	P-value
Urinary GAG (mg/mL)	5	415.8±340.6	69.7±55.3	−345.9±352.5	0.043
Liver volume (cc)	5	1,087.9±329.1	786.5±207.6	−301.5±184.6	0.043
Spleen volume (cc)	5	196.5±71.6	184.2±47.1	−24.3±26.3	0.043
6-Min Walk test (m)	3	519.7±92.7	629.0±139.8	109.3±82.1	0.109
Forced vital capacity (L)	3	1.4±0.5	2.4±0.4	0.9±0.6	0.157
LVMI (g/m^2)	5	64.0±26.8	55.9±23.7	−8.1±9.7	0.042
Shoulder flexion (degrees)	5	86.8±25.5	110.0±15.9	23.2±10.5	0.043
Shoulder abduction (degrees)	5	62.4±20.6	79.2±20.5	16.8±2.4	0.039
Knee flexion (degrees)	5	100.0±12.0	109.0±15.2	9.0±4.6	0.043
Elbow flexion (degrees)	5	110.8±22.2	125.0±26.7	14.2±4.0	0.042
Growth velocity (cm/year)	4	3.7±1.4	7.3±1.5	3.7±0.7	0.068
Respiratory distress index (RDI)	5	10.4±3.1/hour	7.4±2.3/hour	−3±1.2/hour	0.041

Abbreviations: GAG, glycosaminoglycan; m, meters; Left ventricular mass index, LVMI; NA, not applicable.
Note: All values are the observed means±SEM. The change from baseline was analyzed with a Wilcoxon signed-rank test.

mutations, one small insertion mutation, and two *IDS*-*IDS2* recombination mutations (Fig. 1). Of these mutations, 2 are novel (1 small insertion mutation: p.Thr409Hisfs*22, and 1 missense mutation: p.Gly134Glu). Even if *In vitro* functional analysis for a novel missense variant could not be performed in this study, this novel variant has not been detected in more than 100 control alleles and found in dbSNP [http://evs.gs.washington.edu/EVS/]. Conserved sequence elements of glycine residues at position 134 was observed (Fig. 2). The 3D structure of *IDS* containing 134 amino acid residues was determined by X-Ray crystallography at a resolution of 2.0Å (PDB ID:5FQL). The Gly134 residue is close to active site (Fig. 3), suggesting that this mutation might affect electrostatics and catalytic activity. Therefore, it could be assumed that this novel variant is likely to be pathogenic. In patients with the attenuated phenotype (2 of the 3 patients), missense mutations were identified. Two patients with the *IDS*-*IDS2* recombination mutation in this study had severe MPS II phenotypes which was consistent with previous study[13]. The rearrangement mutation identified in our patients involves homologous recombination between intron 3, 7 of the *IDS* gene and the homologous region of its closely related

species	match	aa alignment
Human		134 F K E N G Y V T M S V G K V F H P G I S S N H
mutated	not conserved	134 F K E N G Y V T M S V E K V F H P G I S S N H
Ptroglodytes	no homologue	
Mmulatta	all identical	135 F K E N G Y V T M S V G K V F H P G I T S N H
Fcatus	all identical	132 F K E N G Y V T M S V G K V F H P G I S S N Y
Mmusculus	all identical	136 F K E N G Y V T M S V G K V F H P G I S S N H
Ggallus	all identical	99 F K E N G Y V T M S V G K V F H P G I S S N Y
Trubripes	all identical	126 F K S K G Y F T M S V G K V F H P G I A S N H
Drerio	all identical	128 F K S N G Y T T L S V G K V F H P G I A S

Fig. 2. Conserved sequence elements of glycine residues at position 134 was observed.

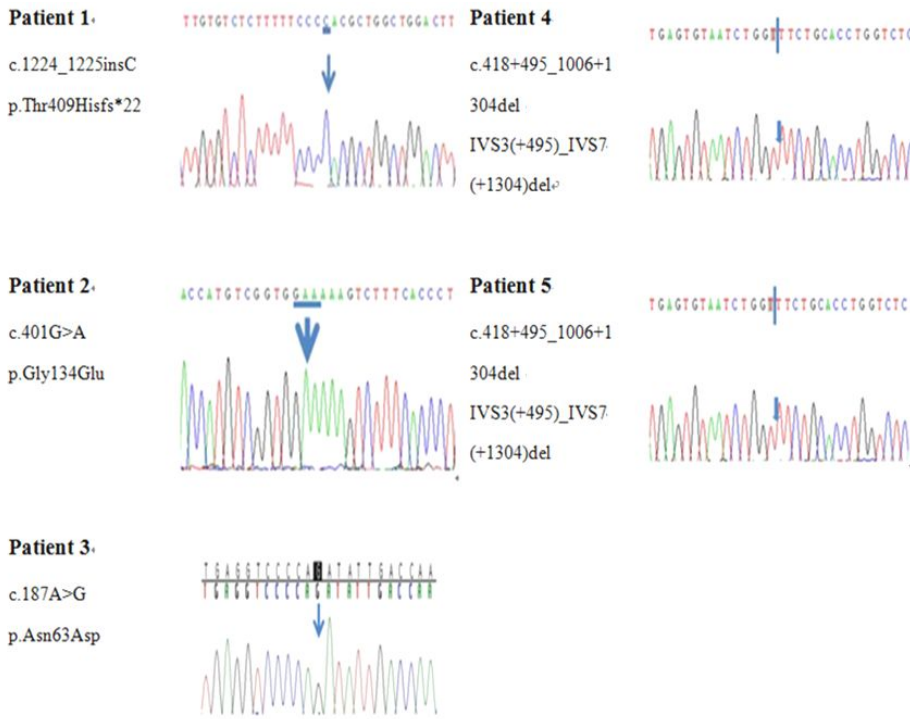

Fig. 1. Partial sequences of *IDS* gene (Patient 1-5) showing the mutations detected in this study.

pseudogene (*IDS2*), and leads to the loss of exons 4, 5, 6, and 7 in genomic DNA. In terms of clinical effectiveness of ERT in patients with MPS II, it has been demonstrated that ERT is helpful in relation to liver and spleen volumes, functional capacity (distance walked in six minutes and forced vital capacity), and urine GAG excretion in patients with MPS II compared with placebo[14]. However, there is no available evidence in the literature on outcomes such as improvement in cardiac function, sleep apnea, quality of life and mortality[14]. In the present study, significant improvements were noted in LVMI and sleep apnea. On the other hand, ERT may have limited effects on the central nervous system (CNS) and skeletal system because of limited enzyme uptake across the blood-brain barrier[15]. The brain atrophy in patient 2 does not show significant progression through the different MR examination, suggesting that ERT therapy does not seems to improve the appearance of neurological imaging findings. Brain atrophy usually develops earlier in MPS II, becoming visible during the first few years of life. A strong correlation was found between severity of brain atrophy and cognitive impairment in MPS [16,17], whereas other authors did not find the same correlation[18]. Also, patients 4 and 5 with severe forms of the disease did not show improvement of neurological deficit in spite of 12 months of ERT. Sohn et al.[19] reported that continuous intrathecal infusion of the deficient enzyme was effective in improving CNS defects in the MPS II mice. Additional study on the continuous intrathecal infusion of the drugs in clinical settings is required.

In conclusion, several clinical improvements in patients with MPS after 12 months of ERT, including liver and spleen volumes, LVMI, RDI, ROM, and urine GAG excretion, were observed without infusion-related complications. Further researches are needed to acquire more information on the long-term effectiveness and safety of ERT due to the short-term period and small numbers in this cohort.

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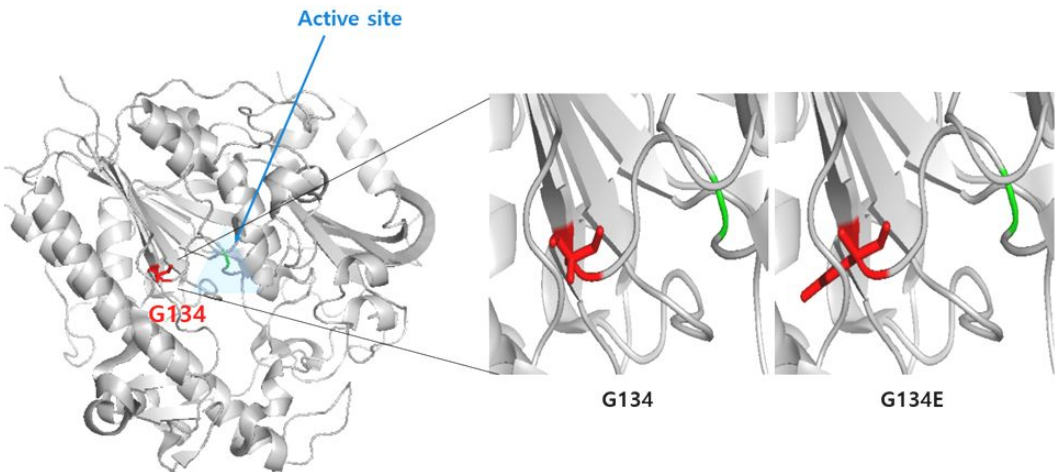

Fig. 3. The 3D structure of IDS containing 134 amino acid residues was determined by X-Ray crystallography at a resolution of 2.0 (PDB ID:5FQL). The Gly134 residue (red color) is close to active site (blue triangle). Conformational change was shown by the p.Gly134 Glu mutation.

요 약

목적: 5명의 제2형 뮤코다당증 환자들의 임상적 스펙트럼과 효소대치요법의 단기간 치료 효과에 관해 알아 보고하고자 하였다.

방법: 5명의 환자들은 임상적 소견, 효소활성화 및 유전자검사에 의해 제2형 뮤코다당증으로 진단되었다. 이두설파제는 일주일 간격으로 0.5 mg/kg의 용량으로 정맥주사 주입을 하였으며, 효소대치요법 시작 전 후 12개월 이상 전신평가를 하였으며, 의무기록을 후향적으로 분석하였다.

결과: 3명의 환자들은 경증 유형, 2명의 환자들은 중증 유형의 제2형 뮤코다당증으로 진단되었다. 진단 시 중위연령은 9.6세(범위 3.4-26세)였다. 네 가계 중 다섯 명의 환자에서 4개의 서로 다른 유전자변이가 확인되었으며, 이중 두 개의 변이는 새로운 돌연변이였다(1개의 작은 삽입돌연변이: p.Thr409Hisfs*22, 1개의 과오돌연변이: p.Gly134Glu). 이중 동일한 유전자돌연변이를 지닌 두 명의 중증 유형의 형제 환자들은 서로 다른 임상적 특징들을 보였다. 12개월 간의 효소대치요법 후 소변 글리코사미노글리칸 배출은 유의하게 감소하였다(P=0.043). 간 및 비장의 용적은 모든 환자에서 유의하게 감소하였다(각각 P=0.043, P=0.043). 이외에도 좌심실질량지수(P=0.042), 어깨관절굽힘각도(P=0.043), 어깨관절벌림각도(P=0.039), 무릎관절굽힘각도(P=0.043), 팔꿈관절굽힘각도(P=0.042), 호흡장애지수(P=0.041)가 모두 호전된 소견을 보였다.

결론: 한국인 제2형 뮤코다당증 환자들은 임상적으로 다양한 특징을 보이며, 단기간의 이두설파제 치료는 주사주입관련 이상반응 없이 심장크기, 호흡장애지수를 포함한 여러 임상적 지표들의 호전에 효과적이었다.

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