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BRIEF REPORT

# Pilot Trial on the Effect of 5-Aminolevulinic Acid on Glucose Tolerance in Patients with Maternally Inherited Diabetes and Deafness

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## ABSTRACT

***Introduction***: The amino acid 5-aminolevulinic acid (5-ALA) is the first heme biosynthetic precursor. The combination of 5-ALA with sodium ferrous citrate (SFC) enhances heme production, leading to increased adenosine triphosphate (ATP) production in mitochondria. We investigated whether administering 5-ALA/SFC improves glucose tolerance with an increase in insulin secretion in patients with maternally inherited diabetes and deafness (MIDD), which is characterized by an insulin secretory disorder due to impaired mitochondrial ATP production.
***Methods***: This was a single-arm, open-label, interventional study. We prospectively administered the oral glucose tolerance test (OGTT) twice in five patients with MIDD who had received intensive insulin therapy: before and 24 weeks after an administration of 5-ALA/SFC (200/232 mg per day). We measured the concentrations of glucose, insulin, C-peptide, and proinsulin at fasting, and 30, 60, and 120 min after glucose load in each OGTT. The primary endpoint was the changes in the area under the curve (AUC) of serum insulin from 0 to 120 min during OGTT from baseline to 24 weeks.
***Results***: The serum insulin AUC (μU/mL) during the 120-min OGTT tended to increase from baseline to 24 weeks but not significantly (17.1 ± 13.7 versus 22.3 ± 13.4, $p = 0.077$). The plasma glucose AUC (mg/dL) during the 120-min OGTT at 24 weeks was not significantly decreased; the late phase of glucose excursion from 60 to 120 min was significantly decreased compared with baseline (357 ± 42 versus 391 ± 50, $p = 0.041$). The mean level of glycated hemoglobin (HbA1c) decreased from 8.3 ± 1.2% at baseline to 7.9 ± 0.3% at 24 weeks ($p = 0.36$) without increasing the daily dose of insulin injections.
***Conclusion***: The 24-week administration of 5-ALA/SFC did not demonstrate a significant improvement in insulin secretion in patients with MIDD. Further investigations with a larger number of patients and a placebo control group are required to clarify the potential efficacy of 5-ALA/SFC for ameliorating mitochondrial dysfunctions in MIDD.
***Trial Registration***: UMIN-CTR000040581 and jRCT071200025.

**Keywords:** 5-Aminolevulinic acid; 5-ALA; Maternally inherited diabetes and deafness; Mitochondria; Mitochondrial diabetes mellitus

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**Key Summary Points**

***Why carry out this study?***

The combination of 5-aminolevulinic acid (5-ALA) with sodium ferrous citrate (SFC) acts on the mitochondrial electron transport chain and improves mitochondrial function.

Administering 5-ALA/SFC improves glycemic control in both patients with prediabetes and those with type 2 diabetes, but the efficacy in patients with maternally inherited diabetes and deafness (MIDD) has not been investigated.

This study aimed to examine our hypothesis that the administration of 5-ALA/SFC could improve insulin secretion in patients with MIDD.

***What was learned from this study?***

The 5-ALA/SFC treatment did not demonstrate a statistically significant improve in insulin secretion but significantly suppressed the late phase of glucose excursion during OGTT in patients with MIDD.

Further investigations with a larger number of patients and a placebo control group are required to clarify whether the administration of 5-ALA/SFC may be a novel and effective adjunctive treatment for MIDD by ameliorating mitochondrial dysfunctions in pancreatic β cells and insulin-targeted organs.

## INTRODUCTION

Mitochondrial diseases are heterogeneous disorders characterized by defects of adenosine triphosphate (ATP) production via oxidative phosphorylation, caused by mutations in the nuclear deoxyribonucleic acid (DNA) and mitochondrial DNA (mtDNA) that encode structural mitochondrial proteins or proteins involved in mitochondrial function [1]. Diabetes mellitus is the most frequent endocrine dysfunctions observed in genetic mitochondrial diseases. The m.3243A > G mutation is the most common point mutation of mtDNA observed in patients with mitochondrial diabetes mellitus (MDM). MDM is characterized by maternal inheritance and progressive neurosensory deafness, and it has also been referred to as maternally inherited diabetes and deafness (MIDD) [2–4]. An impairment of mitochondrial ATP production in pancreatic β cells is considered the main cause of the insulin secretory dysfunction in individuals with MIDD [5, 6], and these individuals require insulin replacement therapy early after diagnosis [7].

5-Aminolevulinic acid (5-ALA), a non-proteinogenic δ amino acid synthesized in mitochondria, is a precursor of heme metabolites. The addition of ferrous iron to 5-ALA enhances the biosynthesis of heme. Heme acts as a protein-bound prosthetic group in mitochondrial respiratory chain complexes II, III, and IV, and cytochrome c. The administration of 5-ALA combined with sodium ferrous citrate (SFC) was reported to enhance heme production and upregulate respiratory chain complexes, leading to an improvement of mitochondrial function [8–11].

Treatment with 5-ALA/SFC might be effective to increase insulin secretion by improving mitochondrial function in β cells. In fact, several studies have shown beneficial effects of 5-ALA/SFC on glycemic control in both patients with prediabetes and those with type 2 diabetes [12–15]. However, the efficacy of 5-ALA/SFC in individuals with MIDD has not been studied. Unless any treatments are administered, most individuals with MIDD eventually require intensive insulin therapy because of a progressive and irreversible decline in their ability to secrete insulin toward a nearly absolute deficiency. We speculated that an administration of 5-ALA/SFC might mitigate and slow the progression of mitochondrial dysfunction of β cells in patients with MIDD.

In this study, we prospectively investigated whether the administration of 5-ALA/SFC could

**Table 1** Time schedule of the study

| Study phase | Screening | Treatment period | | | | |
|---|---|---|---|---|---|---|
| | | Week 0 | Week 4 | Week 8 | Week 16 | Week 24 |
| Informed consent | ● | – | – | – | – | – |
| Enrollment | – | ● | – | – | – | – |
| Baseline characterization check | – | ● | – | – | – | – |
| Confirmation of medication status | – | – | ● | ● | ● | ● |
| Adverse events | – | ● | ● | ● | ● | ● |
| Vital signs | – | ● | ● | ● | ● | ● |
| Required amount of daily insulin treatment | – | ● | ● | ● | ● | ● |
| Laboratory tests | | | | | | |
| Hematology | – | ● | ● | ● | ● | ● |
| Blood chemistry | – | ● | ● | ● | ● | ● |
| Urinalysis | – | ● | ● | ● | ● | ● |
| 75 g OGTT | – | ● | – | – | – | ● |

*OGTT* oral glucose tolerance test
The information was adapted from the study protocol report [16]

improve insulin secretion in patients with MIDD. We also sought to identify what factor(s) determined the efficacy of 5-ALA/SFC treatment for the improvement of glycemic control in patients with MIDD.

# METHODS

## Patients

We recruited Japanese adults diagnosed with MIDD who satisfied all of the following criteria: (1) had a maternal family history of diabetes; (2) required multiple injections of insulin treatment due to a progressive insulin secretory disorder; (3) complicated by progressive neurosensory deafness; and (4) other causes of diabetes were excluded. Patients with porphyria, hemochromatosis, viral hepatitis, and pregnant or lactating women were excluded. Patients who had previously taken 5-ALA were also excluded.

## Study Design

The details of the study design were published in the study protocol report [16]. The present study was a single-arm, open-label, interventional study carried out at Nagasaki University Hospital from August 2020 to October 2021. The study was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR 000040581) and with the Japan Registry of Clinical Trials (jRCT 071200025).

The time schedule of the study is presented in Table 1. We recruited five outpatients with MIDD who were receiving intensive insulin therapy with the FreeStyle Libre intermittently scanned continuous glucose monitoring (isCGM) system (Abbott, Chicago, IL, USA), which is also called a flash glucose monitoring system. The patients were each orally administered 5-ALA 200 mg plus SFC 232 mg daily (5-ALA/SFC 100/116 mg twice a day) for 24 weeks. During the study period, the use of any

antidiabetic medications other than insulin was prohibited.

To evaluate the changes in glucose-stimulated insulin secretion from baseline to the end of 24 weeks of the treatment with 5-ALA/SFC, a 75-g oral glucose tolerance test (OGTT) was administered twice to each patient: before and at 24 weeks after the initiation of 5-ALA/SFC. Both OGTTs were carried out under the condition of overnight fasting. For each OGTT, the patients' insulin and 5-ALA/SFC treatment were provided until the day preceding the OGTT.

The primary endpoint of the study was the changes in the area under the curve (AUC) of serum insulin levels from fasting (0 min) to 120 min during the OGTT from baseline to 24 weeks. The secondary endpoints were the changes in glycated hemoglobin (HbA1c), glycated albumin, the values obtained from the OGTTs, the values obtained from the isCGM system, and the required daily dose of insulin from baseline to 24 weeks. Four weeks of isCGM data were analyzed before initiating 5-ALA/SFC treatment and at 24 weeks after the treatment. For the CGM metrics, time in range (TIR), time below range (TBR), and time above range (TAR) were defined as the percentage of time spent within glucose levels (mg/dL) of 70–180, $< 70$, and $> 180$, respectively. We also examined TBR separately for level 1 (54–69 mg/dL) and level 2 ($< 54$ mg/dL), and TAR separately for level 1 (181–250 mg/dL) and level 2 ($> 250$ mg/dL). As safety endpoints, all adverse events that occurred during the trial were recorded.

### Laboratory Measurements

OGTTs were carried out using a 75-g glucose formulation, Trelan-G75 (AY Pharma, Tokyo). For each OGTT, the levels of plasma glucose (mg/dL), serum insulin (μU/mL), serum C-peptide (ng/mL), and serum proinsulin (pmol/L) were measured at fasting (0 min) and at 30, 60, and 120 min after the ingestion of the glucose load.

The levels of insulin and C-peptide were measured by the ECLusys kit (Roche, Basel, Switzerland). The levels of proinsulin were measured by sandwich enzyme-linked immunosorbent assay (ELISA) kits (Mercodia, Uppsala, Sweden).

### Ethics

The study was approved by the Certified Review Board of Nagasaki University Hospital (no. CRB20-012). We conducted the study in accordance with the Helsinki Declaration of 1964 and its later amendments. Written informed consent was obtained from all participants.

### Statistical Analyses

The values are presented as the mean ± standard deviation (SD) in cases of normal distribution; otherwise, as the medians plus 25th and 75th percentiles. For continuous variables, the paired *t*-test was used to test differences between data obtained at baseline and at 24 weeks after the initiation of 5-ALA/SFC treatment. A repeated-measures analysis of variance (ANOVA) was used to test differences in the values of glucose and hormones during the OGTT between baseline and at 24 weeks after the start of 5-ALA/SFC treatment. The statistical analyses were carried out using JMP Pro 15 (SAS Institute, Cary, NC, USA). $p$-Values $< 0.05$ were considered significant.

## RESULTS

A total of four women and one man participated in the study; their clinical characteristics are summarized in Table 2. All five patients satisfied the criteria of MIDD described above in the Patients and Methods section. All of the patients had at least one of the other characteristics of systemic mitochondrial disease, i.e., muscle weakness, intellectual disability, cerebellar ataxia, cardiomyopathy, short stature, increased level of serum lactate, or neurodegenerative disorders shown by bilateral abnormal lesions in the basal ganglia and brainstem. The m.3243A > G mutation was found in peripheral blood leukocytes in four of the patients (case 5 was the exception). All of the patients completed the study without any

**Table 2** Baseline characteristics of the five patients with MIDD

| | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 |
|---|---|---|---|---|---|
| Sex | M | F | F | F | F |
| Age, years | 47 | 56 | 33 | 60 | 32 |
| Age at diagnosis of diabetes, years | 42 | 25 | 16 | 29 | 30 |
| Duration of diabetes, years | 5 | 31 | 17 | 31 | 2 |
| Height, cm | 162.0 | 150.9 | 153.1 | 147.1 | 148.3 |
| Weight, kg | 47.5 | 38.5 | 46.4 | 37.7 | 37.5 |
| BMI, $kg/m^2$ | 18.1 | 17.0 | 19.8 | 17.4 | 17.1 |
| Total insulin, units/day | 31 | 33 | 37 | 26 | 22 |
| Basal insulin, units/day | 11 | 10 | 11 | 11 | 6 |
| Bolus insulin, units/day | 20 | 23 | 26 | 15 | 16 |
| HbA1c, % | 10.4 | 8.0 | 8.1 | 8.0 | 7.2 |
| HbA1c, mmol/mol | 90 | 63 | 65 | 63 | 55 |
| Glycated albumin, % | 26.0 | 21.2 | 21.6 | 22.8 | 19.8 |
| Fasting C-peptide, ng/mL | 0.13 | 0.41 | 0.67 | 0.24 | 1.75 |
| C-peptide index | 0.10 | 0.19 | 0.30 | 0.11 | 1.24 |
| Insulinogenic index | 0.015 | 0.144 | 0.011 | 0.015 | 0.119 |
| HOMA-IR | 0.21 | 0.85 | 1.64 | 0.43 | 2.37 |
| Matsuda index | 30.54 | 8.78 | 7.90 | 26.52 | 4.30 |
| Diabetic neuropathy | − | + | + | + | + |
| Diabetic retinopathy | − | − | − | + | − |
| Diabetic nephropathy | − | − | − | − | − |
| Maternal family history of diabetes | + | + | + | + | + |
| Family history of mitochondrial disease | + | + | + | + | + |
| Neurosensory deafness | + | + | + | + | + |
| Muscle weakness | + | + | − | + | + |
| Intellectual disability | − | + | + | + | − |
| Cerebellar ataxia | + | − | − | + | + |
| Cardiomyopathy | + | + | − | + | − |
| Short stature (< −2.0 SD) | − | − | − | + | + |
| Increased levels of serum lactate | ND | + | ND | + | − |
| Abnormal lesions in brain on CT/MRI | + | + | + | − | + |

**Table 2** continued

| | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 |
|---|---|---|---|---|---|
| The m.3243A > G mutation in blood leukocytes | + | + | + | + | – |

*BMI* body mass index, *HOMA-IR* homeostasis model assessment-insulin resistance, *ND* no data, *SD* standard deviation
The C-peptide index was calculated as 100 × fasting C-peptide (ng/mL)/fasting glucose (mg/dL). The formulas for the insulinogenic index [17], HOMA-IR [18], and Matsuda index [19] are described in the respective references

severe adverse events. One patient experienced abdominal discomfort during the first few days of taking 5-ALA/SFC but recovered shortly without any medical treatment or interruption of 5-ALA/SFC.

The results of the OGTTs performed before and after the 5-ALA/SFC treatment in each patient are depicted in Fig. 1. At 24 weeks after the initiation of 5-ALA/SFC, four patients (cases 1–4) showed an improvement in glucose levels, with increases in the secretory responses of insulin, C-peptide, and proinsulin. Case 5 did not show any improvement in glycemic or insulin responses.

The mean values from the OGTTs of the five patients before and at 24 weeks of 5-ALA/SFC treatment are shown in Fig. 2. Regarding the primary endpoint of the study, the AUC of serum insulin from 0 to 120 min (AUC $\text{insulin}_{0\text{–}120\ \text{min}}$, μU/mL) during the OGTT tended to increase from baseline to 24 weeks, but not significantly ($17.1 \pm 13.7$ versus $22.3 \pm 13.4$, $p = 0.077$) (Fig. 2f).

Concerning the secondary endpoints, we additionally analyzed the data of OGTTs, isCGM, and clinical features by post hoc decisions. In the OGTT at 24 weeks, the plasma glucose levels at each time point tended to decrease, while the serum levels of insulin, C-peptide, and proinsulin at each time point tended to increase compared with those at baseline; however, the differences were not significant (Fig. 2a–d). The ratios of proinsulin to insulin (or C-peptide) at each time point during the OGTT were comparable between baseline and 24 weeks (data not shown).

The AUC of plasma glucose excursion from 0 to 120 min (AUC $\text{glucose}_{0\text{–}120\ \text{min}}$) during the OGTT at 24 weeks was not significantly different from that measured at baseline. However, the late phase of glucose excursion determined by AUC $\text{glucose}_{60\text{–}120\ \text{min}}$ (mg/dL) at 24 weeks was significantly decreased compared with baseline ($357 \pm 42$ versus $391 \pm 50$, $p = 0.041$) (Fig. 2e). The AUCs of C-peptide and proinsulin also increased from baseline to 24 weeks, but not significantly (Fig. 2g, h).

The results of the comparisons of the laboratory and isCGM data between baseline and 24 weeks after the initiation of 5-ALA/SFC treatment are presented in Table 3. The mean level of HbA1c tended to decrease from $8.3 \pm 1.2\%$ at baseline to $7.9 \pm 0.3\%$ at 24 weeks ($p = 0.36$), even though the required dosage of insulin did not change. Figure 3 shows the time course of mean HbA1c levels during 24 weeks, but the levels of each time point were not significantly different. The data obtained from the isCGM system, including the percentages of time in below/in/above the range of glucose levels, the 24-h mean glucose levels, the SD of glucose levels, and the mean amplitude of glycemic excursions (MAGE) were not significantly different between the baseline and at 24 weeks of 5-ALA/SFC.

## DISCUSSION

We investigated the efficacy of 5-ALA/SFC for improving the glucose tolerance and glucose-stimulated insulin responses of five patients with MIDD by examining the patients' results on 75-g OGTTs administered before and at 24 weeks after the start of treatment with 5-ALA/SFC. The efficacy of 5-ALA/SFC on glucose metabolism has been reported in both patients with prediabetes [12, 13] and those

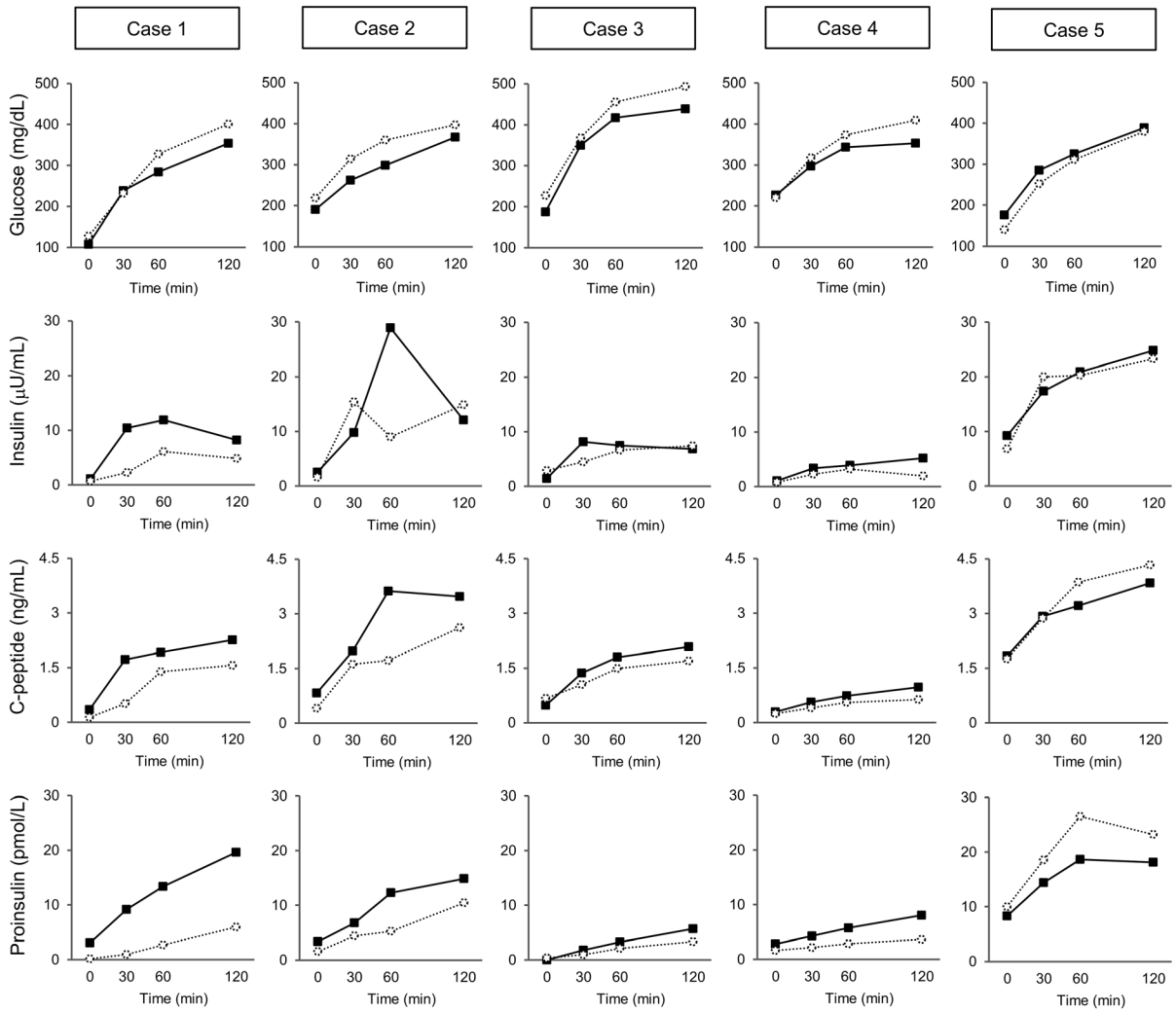
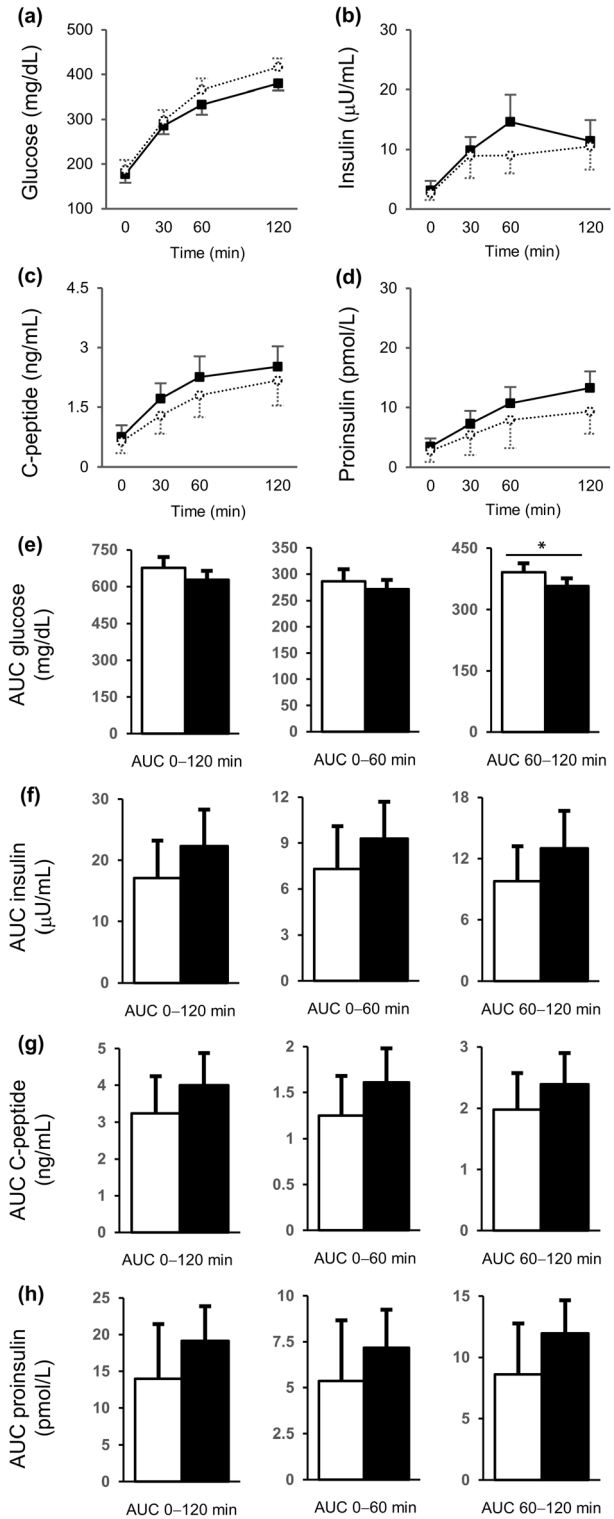



**Fig. 1** The levels of glucose, insulin, C-peptide, and proinsulin during the 75-g OGTTs at baseline and at 24 weeks after the initiation of daily 5-ALA/SFC treatment in each patient with MIDD. Open circles with dotted lines: the values at baseline. Closed squares with solid lines: the values at 24 weeks after the start of the 5-ALA/SFC regimen

with type 2 diabetes with preserved endogenous insulin secretory capacity [14, 15]. To the best of our knowledge, this was the first study to investigate the efficacy of 5-ALA/SFC in patients with MIDD with impaired capacity of insulin secretion.

The primary endpoint of the study (increase in means of AUC insulin $_{0–120\ min}$ in OGTT) could not achieve statistical significance. This study does not adequately support the use of 5-ALA/SFC for the treatment of MIDD. The increase in insulin secretion after 5-ALA/SFC treatment in MIDD, although present in four of the five patients, is of very limited value. We observed that 5-ALA/SFC treatment improved the patients' glycemic profiles, especially during the late phase (60–120 min) of glucose excursion during the OGTT.

We also observed a tendency for an increase in the secretion of proinsulin, a precursor of insulin peptide, in the patients after the 24-week treatment with 5-ALA/SFC. As hyperproinsulinemia reflects a condition in which insulin is not sufficiently processed and a


(a)
Glucose (mg/dL)
500
400
300
200
100
0 30 60 120
Time (min)
(b)
Insulin (μU/mL)
30
20
10
0
0 30 60 120
Time (min)
(c)
C-peptide (ng/mL)
4.5
3
1.5
0
0 30 60 120
Time (min)
(d)
Proinsulin (pmol/L)
30
20
10
0
0 30 60 120
Time (min)
(e)
AUC glucose (mg/dL)
750
600
450
300
150
0
AUC 0–120 min
350
300
250
200
150
100
50
0
AUC 0–60 min
450
300
150
0
*
AUC 60–120 min
(f)
AUC insulin (μU/mL)
30
20
10
0
AUC 0–120 min
12
9
6
3
0
AUC 0–60 min
18
12
6
0
AUC 60–120 min
(g)
AUC C-peptide (ng/mL)
5
4
3
2
1
0
AUC 0–120 min
2
1.5
1
0.5
0
AUC 0–60 min
3
2
1
0
AUC 60–120 min
(h)
AUC proinsulin (pmol/L)
25
20
15
10
5
0
AUC 0–120 min
10
7.5
5
2.5
0
AUC 0–60 min
15
10
5
0
AUC 60–120 min

◀**Fig. 2** Comparisons of the values in 75-g OGTTs between baseline and at 24 weeks after the start of 5-ALA/SFC in patients with MIDD ($n = 5$). Bar: standard error. $^{*}p < 0.05$ between baseline and 24 weeks. Open circles with dotted lines (**a**–**d**) and white bars (**e**–**h**): the values at baseline. Closed squares with solid lines (**a**–**d**) and black bars (**e**–**h**): the values at 24 weeks after the start of 5-ALA/SFC treatment. *AUC* area under the curve

premature release of proinsulin increases, the elevated ratio of proinsulin to insulin (or C-peptide) is considered an indicator of β-cell dysfunction [20]. We did not observe any increases in the ratio of proinsulin to insulin or the ratio of proinsulin to C-peptide from baseline to 24 weeks in the patients. This might indicate that 5-ALA/SFC treatment did not lead to β-cell exhaustion in the patients.

As described above, the effect of 5-ALA/SFC treatment on glucose tolerance varied among the patients; four (cases 1–4) showed an improvement in glucose levels, with a certain degree of insulin increase, while case 5 did not. The m.3243A > G mutation in leukocytes was not detected in case 5 only. The presence of the m.3243A > G mutation in leukocytes might be associated with the glycemic responses to 5-ALA/SFC treatment in patients with MIDD. However, it was reported that only 13.1% of a series of Japanese patients with MIDD were positive for the m.3243A > G mutation in leukocytes [4]. It is considered that mitochondrial dysfunction in pancreatic β cells is not necessarily reflected by the percentage of the m.3243A > G mutation in leukocytes, because the heteroplasmy of mitochondrial DNA shows large variations in distribution among individual tissues [21, 22].

Cases 1–4 showed a similar improvement in glucose levels during the OGTT by 5-ALA/SFC treatment, even though the improvement in insulin secretion was extremely different in each patient. Cases 1 and 2 showed a > 1.5-fold increase in $AUC\ insulin_{\ 0\text{–}120\ min}$ during the OGTT at 24 weeks compared with baseline, whereas cases 3 and 4 showed only slight increases in insulin secretion. Cases 3 and 4 might have achieved improved glucose tolerance mainly via a decrease in insulin resistance in the 24 weeks of 5-ALA/SFC treatment. We could not precisely determine insulin resistance by HOMA-IR and Matsuda index before and after 5-ALA/SFC treatment, because the patients were treated with basal insulin during OGTTs.

Mitochondrial dysfunction in MIDD is thought to result in not only decreased insulin secretion from β cells, but also decreased insulin sensitivity in target organs such as the adipose tissue, skeletal muscle, and liver [23–25]. It was reported that impairments of fatty acid metabolism and insulin signaling developed due to an excessive generation of reactive oxygen species in mitochondria caused insulin resistance [26, 27]. Imeglimin, a novel therapeutic agent for type 2 diabetes with a mechanism that may ameliorate mitochondrial dysfunction, was recently approved in Japan and has been shown to improve both insulin secretion and insulin resistance [28, 29]. Like imeglimin, 5-ALA/SFC might have dual effects on glucose metabolism in patients with MIDD. It was reported that aged 5-ALA synthase 1 gene heterozygous ($ALAS1^{\pm}$) mice developed insulin resistance, with decreased levels of mitochondrial electron transport chain (ETC) complex III subunit, and the treatment of 5-ALA improved their glucose tolerance with increased insulin sensitivity and the complex III protein levels [9]. The administration of 5-ALA/SFC to aged sarcopenic mice improved their glucose tolerance and increased muscle mass with enhanced gene expression of mitochondrial ETC complex IV in muscles [10]. Both imeglimin and 5-ALA/SFC act on the mitochondrial ETC, but their targets are different. Imeglimin partially inhibits mitochondrial ETC complex I and restores the function of complex III [30, 31], whereas 5-ALA/SFC mainly restores the functions of the complexes III and IV [8–11]. It remains unclear how the differences in their actions for mitochondria affect glucose metabolism.

In the present study, no severe adverse events (including hypoglycemia) were observed in the patients during the 24 weeks of 5-ALA/SFC treatment. As was already reported, the safety and tolerability of 5-ALA/SFC could be considered advantageous in the treatment of patients with any type of diabetes [12–15, 32].

**Table 3** Comparison of data at baseline and after 24 weeks of 5-ALA/SFC regimen

| | Baseline | 24 weeks | *p*-Value |
|---|---|---|---|
| Male/female, *n* | 1/4 | | |
| Age, years | 45.6 ± 12.9 | | |
| Duration of diabetes, years | 17 (4–31) | | |
| Height, cm | 152.3 ± 5.9 | | |
| Weight, kg | 41.5 ± 5.0 | 42.6 ± 5.6 | 0.14 |
| BMI, kg/m$^2$ | 17.9 ± 1.2 | 18.3 ± 1.3 | 0.15 |
| Total insulin, units/day | 29.8 ± 5.9 | 29.0 ± 5.7 | 0.24 |
| Basal insulin, units/day | 9.8 ± 2.2 | 9.4 ± 2.1 | 0.18 |
| Bolus insulin, units/day | 20.0 ± 4.6 | 19.6 ± 4.4 | 0.48 |
| Glucose parameters detected by isCGM: | | | |
| TAR (> 180 mg/dL), % | 37.9 ± 17.1 | 39.2 ± 13.7 | 0.89 |
| TAR Level 2 (> 250 mg/dL), % | 10.3 ± 12.3 | 8.0 ± 5.3 | 0.59 |
| TAR Level 1 (181–250 mg/dL), % | 27.7 ± 6.6 | 31.2 ± 11.4 | 0.55 |
| TIR (70–180 mg/dL), % | 60.8 ± 17.1 | 60.0 ± 13.9 | 0.92 |
| TBR (< 70 mg/dL), % | 1.2 ± 1.8 | 0.9 ± 0.9 | 0.48 |
| TBR Level 1 (54–69 mg/dL), % | 0.9 ± 1.1 | 0.7 ± 0.5 | 0.51 |
| TBR Level 2 (< 54 mg/dL), % | 0.3 ± 0.8 | 0.2 ± 0.4 | 0.46 |
| Mean glucose, mg/dL | 170.2 ± 25.9 | 168.5 ± 12.6 | 0.88 |
| SD of glucose, mg/dL | 53.5 ± 13.4 | 52.4 ± 8.6 | 0.76 |
| MAGE, mg/dL | 127.5 ± 33.3 | 124.7 ± 23.1 | 0.79 |
| HbA1c, % | 8.3 ± 1.2 | 7.9 ± 0.3 | 0.36 |
| HbA1c, mmol/mol | 67.2 ± 13.3 | 62.2 ± 3.7 | 0.36 |
| Glycated albumin, % | 22.3 ± 2.3 | 21.7 ± 1.7 | 0.63 |
| Fasting plasma glucose, mg/dL | 186.8 ± 48.1 | 177.4 ± 43.1 | 0.52 |
| Fasting C-peptide, ng/mL | 0.64 ± 0.65 | 0.76 ± 0.64 | 0.31 |
| LDL-C, mg/dL | 105 ± 33 | 94 ± 21 | 0.16 |
| HDL-C, mg/dL | 63 ± 7 | 59 ± 16 | 0.45 |
| TG, mg/dL | 73 (59–228) | 117 (68–458) | 0.44 |
| UACR, mg/gCr | 26.3 (18.9–28.4) | 22.3 (13.6–91.6) | 0.63 |

Data are mean ± SD or median (25–75% confidence interval) for continuous variables
*BMI* body mass index, *HDL-C* high-density lipoprotein, *isCGM* intermittently scanned continuous glucose monitoring, *LDL-C* low-density lipoprotein, *MAGE* mean amplitude of glycemic excursions, *SD* standard deviation, *TAR* time above range, *TBR* time below range, *TIR* time in range, *TG* triglyceride, *UACR* urine albumin/creatinine ratio
*p*-values for baseline versus 24 weeks in all data were calculated by paired *t*-tests

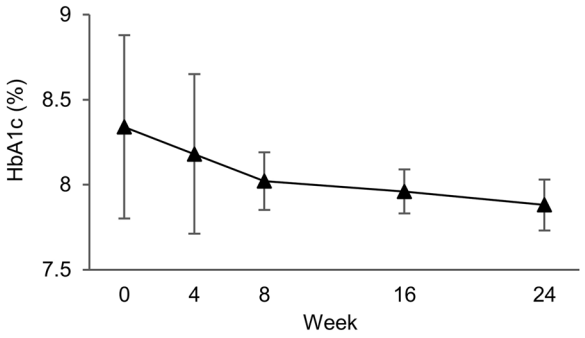



**Fig. 3** Time course of mean HbA1c levels during 24 weeks of 5-ALA/SFC treatment. The HbA1c levels of each time point were not significantly different. Bar: standard error

Our study has several limitations. It was carried out at a single center, and the sample size was small. We could find out only five patients with MIDD among 771 patients with diabetes, including type 1 and 2 diabetes at our hospital, because MIDD is a very rare diabetes and its diagnosis is very difficult to make. We did not prepare a placebo control group. The change in HbA1c over 24 weeks in the five patients with MIDD was similar to common observations of changes in placebo arms of blinded trials of glucose lowering agents. In addition, we had little evidence to determine the dosage of 5-ALA/SFC that was appropriate for patients with MIDD. We prescribed our patients 5-ALA/SFC 200/232 mg per day, because this dosage was confirmed to be safe in a study of patients with type 2 diabetes [15]. Our treatment period of 24 weeks of 5-ALA/SFC might also be insufficient to lead to a definitive conclusion regarding the improvement of insulin secretion and glucose tolerance. Considering the proposed mechanism of action of 5-ALA, it is unlikely that we would not observe an effect on the improvement of glucose metabolism in patients with MIDD.

## CONCLUSIONS

This study did not clearly evidence that treatment with 5-ALA/SFC could improve glucose tolerance and restore insulin secretion in patients with MIDD. However, the efficacy of 5-ALA/SFC was quite different among the five patients with MIDD. Further investigations with a larger number of patients and a placebo control group are required to clarify the potential efficacy of 5-ALA/SFC for ameliorating mitochondrial dysfunctions in pancreatic β cells and insulin-targeted organs in patients with MIDD.

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***Author Contributions.*** A. Haraguchi, I. Horie, and N. Abiru designed the study. Y. Nakamura, A. Haraguchi, and N. Abiru accomplished the study. Y. Nakamura, I. Horie and N. Abiru analyzed the data for the study. Y. Nakamura, A. Haraguchi, I. Horie, A. Kawakami and N. Abiru drafted the manuscript. All authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship of this article, take responsibility for the integrity of the work as a whole, and have approved the publication of this version of the manuscript.

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***Disclosures.*** Y. Nakamura, A. Haraguchi, I. Horie, A. Kawakami, and N. Abiru declare that

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***Compliance with Ethics Guidelines.*** The study was approved by the Certified Review Board of Nagasaki University Hospital (no. CRB20-012). We conducted the study in accordance with the Helsinki Declaration of 1964 and its later amendments. A written informed consent was obtained from all participants.

***Data Availability.*** The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.



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