Original Article

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A phase II investigator-initiated, continuation trial of 5-aminolevulinic acid hydrochloride/sodium ferrous citrate (5-ALA-HCl/SFC) for patients with adult-onset Still's disease (AOSD) refractory to glucocorticoids

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SUMMARY: The aim of this study is to evaluate the efficacy and safety of 5-aminolevulinic acid hydrochloride/sodium ferrous citrate (5-ALA-HCl/SFC) in patients with adult-onset Still's disease (AOSD) refractory to glucocorticoids during the maintenance treatment period of a phase II investigator-initiated randomized, double-blind, parallel-group clinical trial. This multicenter, open-label, continuous trial was limited to participants from the preceding study, which was planned for 30 enrollees but was stopped early after four enrollees because of slow recruitment. The current trial maintained a 16-week treatment period and remained double-blind until the previous study database was locked and unblinded. Two dose groups were used: 5-ALA-HCl/SFC 100 and 300 mg. Participants previously assigned to placebo were re-randomized 1:1 to either dose group. The dosage and administration were identical to those of the previous protocol. The primary endpoint was the achievement of adapted ACR 30 at week 16. All four participants achieved the primary endpoints. Secondary outcomes, including the adapted ACR 90/100 and the change in serum ferritin levels, showed numerically greater improvement in the participant who received the high-dose compared with the other. Eleven adverse events occurred in three participants, including one serious event and one discontinuation; all were deemed unrelated to the study drug, and no deaths were reported. This study identified potential efficacy signals, particularly in the high-dose group. However, because of premature termination and a very limited sample size, the data were insufficient to establish the efficacy and safety profile of 5-ALA-HCl/SFC in refractory AOSD. Larger studies are required to confirm these findings.

Keywords: Adult-onset Still's disease (AOSD), glucocorticoids, heme oxygenase-1 (HO-1), 5-aminolevulinic acid/sodium ferrous citrate (5-ALA-HCl/ SFC)

1. Introduction

Adult-onset Still's disease (AOSD) is a systemic inflammatory disorder. Autoantibodies, such as antinuclear antibodies, are generally not detected; however, hypercytokinemia involving interleukin (IL)-1β, IL-6, IL-18, and tumor necrosis factor-α (TNF-α) due to abnormal activation of innate immune cells, such as monocytes and macrophages, may be involved in disease pathogenesis (*1*). Although the recent European Alliance of Associations for Rheumatology/Paediatric Rheumatology European Society (EULAR/PReS) guidelines recommend biologics as the first-line treatment (*2*), in Japan, initial therapy often begins with

glucocorticoids (GCs). The prognosis is generally good; however, approximately 60% of patients experience a prolonged disease course. As GCs alone are often insufficient to control the disease, biologic agents targeting IL-6 and IL-1β or immunosuppressants are used in combination. However, these carry the risk of side effects, such as increased susceptibility to infection. Therefore, there is a need to develop new therapeutic drugs with novel mechanisms.

Elevated levels of heme oxygenase-1 (HO-1) have been reported in the sera of patients with AOSD (*3,4*). The dynamics of HO-1 are similar to those of serum ferritin, which is a marker of disease activity in AOSD. The decrease in HO-1 expression with treatment suggests that it may contribute to the pathogenesis of the disease.

5-Aminolevulinic acid (5-ALA) is an amino acid involved in porphyrin metabolism in plants and animals. Within cells, eight molecules of 5-ALA undergo a series of enzymatic reactions to form protoporphyrin IX (PpIX), which is then converted into heme by ferrochelatase through the insertion of ferrous iron. The co-administration of 5-ALA hydrochloride (5-ALA-HCl) with sodium ferrous citrate (SFC) enhances the conversion of PpIX to heme (*5*).

Preclinical studies in Balb/c mice and Sprague-Dawley rats have demonstrated that oral intake of 5-ALA-HCl combined with SFC increases intracellular heme levels, leading to the upregulation of HO-1 expression. HO-1, also known as heat shock protein 32 (HSP32), is a 32-kDa enzyme that degrades heme and exhibits anti-inflammatory properties (*6,7*). It has been shown to be beneficial in conditions such as ischemia-reperfusion injury, cisplatin-induced nephropathy, and various inflammatory disorders (*8, 9*). Importantly, the administration of 5-ALA-HCl alone does not sufficiently induce HO-1 expression or achieve therapeutic benefits in these conditions. Therefore, the combination of 5-ALA-HCl and SFC is considered essential for eliciting a significant anti-inflammatory response (*8-10*).

Based on these findings, we thought that 5-ALA-HCl/SFC could be a potential therapeutic agent for AOSD, we conducted a Phase II study (jRCT2071220040) to confirm its efficacy of 5-ALA-HCl/SFC in patients with AOSD. This study was an extension trial designed to evaluate the efficacy and safety of continuous administration over a 16-week period.

2. Methods

2.1. Study design

This is an investigator-initiated, multicenter, open-label, continuous clinical trial of the efficacy and safety of 5-ALA-HCl/SFC in patients with AOSD who are refractory to GCs. Owing to early termination, this study only presents pilot data from four participants. The small sample size means that the results can only be considered preliminary evidence that requires validation in larger, adequately powered studies.

This study was conducted at 12 centers in Japan and was performed in accordance with the principles of the Declaration of Helsinki (*11*) and Japan Good Clinical Practice. The study was registered in the Japan Registry of Clinical Trials (*https://jrct.mhlw.go.jp*) as jRCT2071220086 and was approved by the Institutional Review Board of Nagasaki University Hospital, Hokkaido University Hospital, Hamamatsu University School of Medicine, University of Tsukuba Hospital, National Hospital Organization Kyushu Medical Center, Osaka Metropolitan University Hospital, Fukushima Medical University Hospital, Okayama Saiseikai Outpatient Center Hospital, Keio University Hospital, Nagoya University Hospital, Yokohama City University Hospital, and Hiroshima University Hospital. Participants were recruited from 25 January 2023 to 1 February 2024. This trial is a continuation of a previous Phase II physician-initiated randomized double-blind parallel group comparative clinical trial (*12*) (jRCT2071220040). The participants were divided into two groups: the 5-ALA-HCl 100 mg/day + SFC 78.4 mg/day (low-dose) group and the 5-ALA-HCl 300 mg/day + SFC 235.2 mg/day (high-dose) group. The rationale for selecting the dosage in this clinical trial was as follows. Previous clinical studies conducted in patients with type 2 diabetes and cisplatin-induced nephropathy demonstrated the efficacy of 5-ALA-HCl at 300 mg/day (150 mg administered twice daily) (trial IDs: NPJ005-DM2-0522 and SPP3C301). The proposed mechanisms of action involve activation of the electron transport chain and induction of HO-1 expression, leading to anti-inflammatory effects in type 2 diabetes and HO-1 induction in cisplatin nephropathy (*8,9,13*). As these mechanisms are thought to overlap with the pathophysiology of AOSD, a dose of 300 mg/day was anticipated to be effective in this indication as well. In addition, because this study was designed as a dose-finding trial, a lower dose group (100 mg/day) was included, resulting in two study arms: 100 mg/day and 300 mg/day.

2.2. Patients

Participants who met all of the following criteria were recruited: (1) patients who had completed the prior study; (2) patients whose free informed consent could be obtained in writing and who were able to comply with the requirements of the study protocol; (3) females of childbearing potential or males who were sexually active with females of childbearing potential who agreed to use an effective contraceptive method (*e.g.*, condom) for the duration of the study and until the day after the last dose of the study drug.

Key exclusion criteria were as follows: (1) females who were lactating, pregnant, or intend to become

pregnant during the study period; (2) patients who have been off study medication for more than 14 consecutive days between the start of the prior study and the start of the continuation study; (3) patients who have been off study medication for more than 7 consecutive days from the week 8 evaluation date of the prior study to the time of enrollment; (4) patients with macrophage activation syndrome (MAS) or disseminated intravascular coagulation syndrome since the start of the prior study; (5) patients who have started a new or increased dose of methotrexate or other disease-modifying anti-rheumatic drugs between the end of the prior study and the time of enrollment; (6) patients with serious complications that make them unsuitable for the study as determined by the investigator or sub-investigator, (7) other patients deemed inappropriate by the investigator or sub-investigator.

2.3. Interventions

This trial was a continuation of a previous Phase II physician-initiated randomized double-blind parallel group clinical trial (*12*) (jRCT2071220040). The study was conducted in two groups: low-dose and high-dose groups. Subjects who were assigned to the placebo group in the previous study were randomly assigned at a 1:1 ratio to either the low-dose or high-dose group at the time of assignment in the previous study. The remaining groups received equivalent dosages and administration as in the previous study. The study drug (5-ALA-HCl/SFC) was provided by KIYAN PHARMA Co., Ltd. It was administered orally twice daily for 16 weeks. The study was conducted under double-blind conditions until the database of the previous study was finalized and unlocked. The target enrollment was 30 participants (10 per group). This calculation was based on the assumption that approximately 550 patients with AOSD would receive outpatient care at the 11 major institutions and their associated facilities that were expected to participate in the trial. According to a national survey of patients with AOSD, 11.6% were deemed by their physicians to have residual disease activity at the final assessment (*14*). Similarly, when the trial was planned, approximately 20% of patients with AOSD at Nagasaki University Hospital were considered to have residual disease activity. In accordance with these figures, it was estimated that around 80 patients (approximately 15% of the total) across the 11 institutions (including affiliated sites) would meet this criterion. In a previous clinical trial (UMIN000028010: randomized, double-blind, parallel-group comparison of tocilizumab for colchicine-resistant familial Mediterranean fever), approximately 40% of the screened patients were successfully enrolled. Based on this experience, it was projected that 30 participants would participate in the present investigator-initiated trial. However, because the study was terminated early, only four patients were enrolled, and their data are presented here as pilot data. Participants who achieved adapted ACR 30 at follow-up visits were eligible for GCs tapering at the physician's discretion. The GCs' tapering regimen was performed in accordance with a predetermined protocol. If an increase or re-increase in oral GCs was considered necessary, the study drug was discontinued. The study drug was also discontinued if the investigator or sub-investigator determined that an adverse event was causally related to the study drug and was serious, medically significant, or unacceptable. This period was limited to a maximum of two consecutive weeks (*i.e.*, 14 days). If no improvement was observed during this period, the study drug was discontinued. Even if the study drug was withdrawn, evaluation and observation were continued as part of the trial.

During the study period, the initiation of the following treatments was prohibited: administration of immunosuppressants, biologics, or Janus kinase inhibitors; intra-articular GC injections at joints; and suppositories.

Participants were discontinued prematurely if they met any of the following criteria: if they asked to leave the trial, if it was determined after registration that they were ineligible for the trial, if an investigator or sub-investigator determined that continuing the trial was difficult due to complications worsening or adverse events, if pregnancy was confirmed, if the entire clinical trial was discontinued, if any other serious violations of the clinical trial protocol were identified, or if the investigator or sub-investigator determined that termination was necessary.

2.4. Study endpoints

The primary endpoint was the achievement of adapted ACR30 (*2,15*) at week 16 following the initiation of 5-ALA-HCl/SFC administration. For participants in the active drug group in the previous study, 5-ALA-HCl/SFC administration was defined as the start date of the previous study. For subjects in the placebo group in the previous study, it was defined as the start date of the current study. Adapted ACR 30 is a composite measure of the disease activity. It was defined as meeting the following criteria: improvement of ≥ 30% in three or more of the following five items 1) to 5) from baseline, with no fever exceeding 38°C in the past week, and no worsening of more than 30% in any of the following five items. 1) Physician-assessed global assessment of disease activity (VAS); 2) Patient-assessed disease activity (VAS); 3) Functional status assessed using the Health Assessment Questionnaire Disability Index (HAQ-DI); 4) Number of active joints (swollen joints and tender joints); 5) Laboratory values of ESR (mm/hr) or CRP (mg/dL).

The secondary endpoints included the following: Adapted ACR 30 at weeks 4, 8, 12, 20, and 24; Adapted ACR 50/ 70/ 90/ 100 at weeks 4, 8, 12, 16, 20, and 24; GCs dose reduction at weeks 4, 8, 12, 16, 20,

and 24; changes in systemic feature scores (SFS) at weeks 4,8, 12, 16, 20,and 24; changes in serum ferritin levels at weeks 8, 16,and 24; and changes in EuroQol 5-dimensions 5-levels (EQ-5D-5L) at weeks 4, 8, 12, 16, 20, and 24. Adverse events were also collected as safety data. For adapted ACR 50/ 70/ 90/ 100, it is defined as meeting the following criteria: three or more of the five items listed in 1) to 5) above have improved by 50%, 70%, 90%, or 100%, there has been no fever exceeding 38°C in the past week, and no more than one item has worsened by more than 30% of the five items listed in 1) to 5) above. The SFS scoring system consists of five clinical and five laboratory assessments and was designed to evaluate systemic disease features (*16*). It has also been used previously as a secondary endpoint in a phase III trial investigating the efficacy of tocilizumab in Japanese patients with AOSD (*17*). Assessments in weeks 20 and 24 were limited to participants who had received the active drug in the prior study.

2.5. Statistical analysis

Although the study initially aimed to enroll 30 participants, it was terminated prematurely, and data from only four participants were available for analysis and were reported as pilot observations. Owing to early discontinuation and the limited sample size, no formal inferential statistical analyses were performed. Instead, descriptive statistics were provided for each patient. Categorical variables are summarized as counts and percentages based on the number of participants analyzed, whereas continuous variables are presented as medians with corresponding minimum and maximum values. All analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Patient flow and characteristics

The trial was conducted from July 28, 2022, to April 30, 2024. Owing to the impact of the COVID-19 pandemic, the recruitment of clinical trial participants did not proceed as planned. Owing to delays in participant enrollment and funding issues, the trial investigator decided to terminate the trial early. Four participants who completed the previous study were enrolled. Figure 1 shows a flowchart of the study. The details are as follows: one participant who was in the placebo group in the previous study was enrolled in the low-dose group, resulting in one participant in the high-dose group and three participants in the low-dose group. Among the three participants in the low-dose group, two discontinued the study drug during the trial period: one due to a worsening of the underlying disease on day 37, and one due to an adverse event and discontinuation of the study drug for more than 14 days on day 71. However, they completed the remaining observation period of the trial after switching to a different treatment.

Table 1 presents the results of the baseline

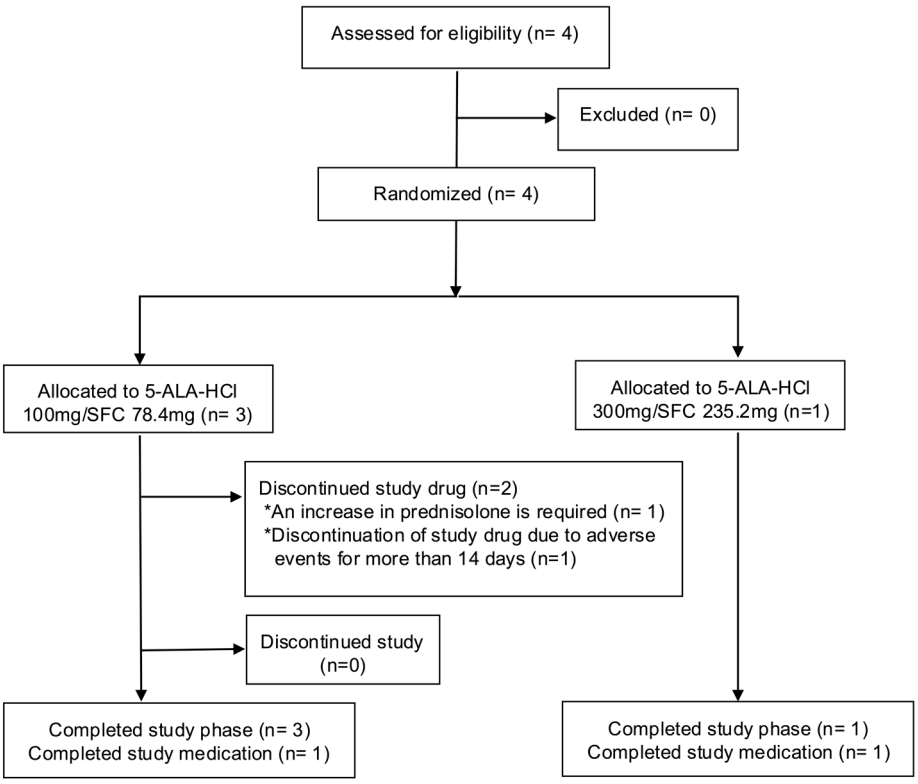

Figure 1. Flowchart of the study.

characteristics of the participants. The median age of the participants was 59.5 years. Two of the four participants were female. The median disease duration was 28.3 months. Of the four participants, three had a history of treatment with drugs other than prednisolone, and one participant (in the low-dose group) had no history of treatment with drugs other than prednisolone. The median dose of concomitant prednisolone was 14 mg/day. The median CRP, ESR, and ferritin levels were 3.23 mg/dL, 37.5 mm/h, and 1819 ng/mL, respectively. The median systemic feature score was 3, and the median EQ-5D-5L score was 0.72.

3.2. Primary endpoint

The primary endpoint, the adapted ACR 30 at week 16 after administration of the study drug, was met by all participants (four participants: one in the high-dose group and three in the low-dose group). However, in two participants in the low-dose group (cases 2 and 4), the study drug was discontinued by week 16 (day 71 and day 37), and week 16 was evaluated under another treatment.

3.3. Secondary endpoints

Table 2 lists the secondary endpoints and the CRP and ESR levels for each case.

3.3.1. Adapted ACR 30 at weeks 4, 8, 12, 20, and 24

The participant in the high-dose group (Case 1) achieved adapted ACR 30 at all time points. One participant in the low-dose group (Case 2) also achieved adapted ACR 30 at all time points; however, the study drug was discontinued on day 71 of this study. Another participant in the low-dose group (Case 3) achieved adapted ACR 30 at week 4 but did not achieve it at weeks 8, 12, 20, or 24. One participant who was in the placebo group in the prior study and enrolled in the low-dose group in this study (Case 4) achieved adapted ACR30 at week 12 only (the study drug was discontinued on day 37 of this study).

3.3.2. Adapted ACR 50/ 70/ 90/ 100 at weeks 4, 8, 12, 16, 20, and 24

One participant in the high-dose group (Case 1) achieved adapted ACR 70 at week 4, adapted ACR 90 at weeks 8, 12, 16, and 24, and adapted ACR 100 at week 20. In the low-dose group, Case 2 achieved adapted ACR 70 at weeks 4, 8, 20, and 24 but did not achieve adapted ACR 50 at week 12 and achieved adapted ACR 50 at week 16 (the study drug was discontinued on day 71). Case 3 achieved adapted ACR 50 at week 4 but did not achieve adapted ACR 50 at any time point after week 8. Case 4 achieved adapted ACR 50 at week 12 but did not achieve adapted ACR 50 at weeks 4, 8, or 16 (the study drug was discontinued on Day 37).

3.3.3. GCs dose reduction

One participant in the high-dose group (Case 1) received a prednisolone dose of 10 mg per day at all time points from baseline to the final observation date. In the low-dose group, Case 2 (baseline dose: 30 mg/day) experienced the changes at weeks 4, 8, 12, 16, 20 and

Table 1. Baseline characteristics of patients with adult onset still's disease treated with study drug (*n* = 4)

Characteristics	Median (min-max) or n (%)	Case 1	Case 2	Case 3	Case 4
Study drug		5-ALA-HCl 300 mg/day + SFC 235.2 mg/day	5-ALA-HCl 100 mg/day + SFC 78.4 mg/day	5-ALA-HCl 100 mg/day + SFC 78.4 mg/day	5-ALA-HCl 100 mg/day + SFC 78.4 mg/day
Age	59.5 (19-77)	77	65	54	19
Sex, female, n (%)	2 (50%)	Female	Male	Male	Female
Body weight, kg	63.4 (44.4-91.4)	44.4	61.8	65	91.4
Disease duration, months	28.3 (7.8-145.2)	48.4	145.2	7.8	8.2
Previous treatment other than prednisolone, n (%)	3 (75%)	Yes	Yes	Yes	No
Tocilizumab, n (%)	1 (25%)		Yes		
Methotrexate, n (%)	1 (25%)		Yes		
Cyclosporine A, n (%)	2 (50%)		Yes	Yes	
Methylprednisolone plus therapy, n (%)	2 (50%)	Yes	Yes		
Dose of concomitant prednisolone, mg	14 (10-30)	10	30	13	15
CRP, mg/dL	3.23 (0.07-7.4)	7.4	2.76	3.69	0.07
ESR, mm/hr	37.5 (9-101)	101	41	34	9
Serum ferritin, ng/mL	1819 (110-4610)	4610	3360	278	110
Systemic feature score	3 (1-5)	5	3	3	1
EQ-5D-5L (score)	0.72 (0.04-0.78)	0.04	0.78	0.68	0.76
EQ-5D-5L (VAS)	62.5 (20-75)	20	50	75	75

CRP: C-reactive protein; EQ-5D-5L: EuroQol 5-dimensions 5-levels; ESR: erythrocyte sedimentation rate; SFC: sodium ferrous citrate; VAS: visual analog scale; 5-ALA: 5-aminolevulinic acid.

Table 2. Primary, secondary endpoints, CRP, and ESR levels

Endpoints	Case 1	Case 2	Case 3	Case 4*
Study drug	5-ALA-HCl 300 mg/day + SFC 235.2 mg/day	5-ALA-HCl 100 mg/day + SFC 78.4 mg/day	5-ALA-HCl 100 mg/day + SFC 78.4 mg/day	5-ALA-HCl 100 mg/day + SFC 78.4 mg/day
At the time of discontinuation of study drug	-	Day 71	-	Day 37
Achieved Adapted ACR values				
Week 4 (Day 29)	70	70	50	Not achieved
Week 8 (Day 57)	90	70	Not achieved	Not achieved
Week 12 (Day 85)	90	30	Not achieved	50
Week 16 (Day 113)	90	50	30	30
Week 20 (Day 141)	100	70	Not achieved	-
Week 24 (Day 169)	90	70	Not achieved	-
Systemic feature score				
Baseline (Day 1)	5	3	3	1
Week 4 (Day 29)	4	3	4	3
Week 8 (Day 57)	2	2	4	2
Week 12 (Day 85)	2	2	3	1
Week 16 (Day 113)	2	2	4	1
Week 20 (Day 141)	2	2	4	-
Week 24 (Day 169)	2	2	4	-
Serum ferritin (ng/mL)				
Baseline (Day 1)	4610	3360	278	110
Week 8 (Day 57)	431	386	431	7863
Week 16 (Day 113)	98	105	431	239
Week 24 (Day 169)	267	188	562	-
EQ-5D-5L scores				
Baseline (Day 1)	0.04	0.78	0.68	0.76
Week 4 (Day 29)	0.89	0.94	0.89	0.71
Week 8 (Day 57)	0.94	0.94	0.76	0.8
Week 12 (Day 85)	0.94	0.94	0.84	0.94
Week 16 (Day 113)	0.94	0.94	0.84	0.88
Week 20 (Day 141)	0.94	0.89	0.84	-
Week 24 (Day 169)	0.82	0.94	0.76	-
EQ-5D-5L (VAS)				
Baseline (Day 1)	20	50	75	75
Week 4 (Day 29)	95	90	90	40
Week 8 (Day 57)	99	90	50	80
Week 12 (Day 85)	99	60	90	85
Week 16 (Day 113)	100	50	70	85
Week 20 (Day 141)	100	60	60	-
Week 24 (Day 169)	80	90	40	-
CRP (mg/dL)				
Baseline (Day 1)	7.4	2.76	3.69	0.07
Week 4 (Day 29)	3.45	0.39	3.88	0.93
Week 8 (Day 57)	1.04	0.31	8.92	0.56
Week 12 (Day 85)	0.27	0.75	1.63	0.009
Week 16 (Day 113)	0.73	1.05	4.67	0.02
Week 20 (Day 141)	0.52	0.009	8.16	-
Week 24 (Day 169)	1.41	0.59	6.55	-
ESR (mm/hr)				
Baseline (Day 1)	101	41	34	9
Week 4 (Day 29)	79	42	31	21
Week 8 (Day 57)	55	47	77	8
Week 12 (Day 85)	41	22	65	2
Week 16 (Day 113)	36	18	69	1
Week 20 (Day 141)	35	12	92	-
Week 24 (Day 169)	77	40	75	-
Prednisolone dose (mg/day)				
Baseline (Day 1)	10	30	13	15
Week 4 (Day 29)	10	25	13	12.5
Week 8 (Day 57)	10	17.5	13	60
Week 12 (Day 85)	10	20	13	50
Week 16 (Day 113)	10	20	13	30
Week 20 (Day 141)	10	30	13	-
Week 24 (Day 169)	10	20	13	-

ACR: American College of Rheumatology; CRP: C-reactive protein; EQ-5D-5L: EuroQol 5-dimensions 5-levels; ESR: erythrocyte sedimentation rate; SFC: sodium ferrous citrate; VAS: visual analog scale; 5-ALA: 5-aminolevulinic acid. *Case 4 is the placebo group of the prior study and only has data up to 16 weeks. All other cases have results up to 24 weeks, using the induction of study drug in the prior study as baseline. The study drug was discontinued for Cases 2 and 4 during the trial, after which the patients were transferred to standard treatment.

24: -5, -12.5, -10, -10, 0 and -10 mg/day. However, the study drug was discontinued on Day 71. Case 3 received a prednisolone dose of 13 mg/day at all time points. Case 4 (baseline dose: 15 mg/day) experienced the changes at weeks 4, 8, 12 and 16: -2.5, +45, +35 and +15 mg/day. However, the study drug was discontinued on Day 37.

3.3.4. Changes in systemic feature scores, serum ferritin, and EQ-5D-5L

Table 2 shows the absolute SFS, absolute serum ferritin levels, absolute EQ-5D-5L index scores and absolute EQ-5D-5L VAS scores for each case at baseline and subsequent visits.

Case 1 (the high dose group) showed SFS changes of -1, -3, -3, -3, -3, -3 at weeks 4, 8, 12, 16, 20, and 24. In the low-dose group, one participant (Case 2) showed SFS changes of 0, -1, -1, -1, -1, -1 at weeks 4, 8, 12, 16, 20, and 24. In another participant (Case 3), the SFS change at weeks 4, 8, 12, 16, 20, and 24 were +1, +1, 0, +1, +1, +1. Case 4 had SFS changes of +2, +1, 0, and 0 at weeks 4, 8, 12, and 16.

We also observed changes in serum ferritin levels. The changes in serum ferritin levels at weeks 8, 16, and 24 in the high-dose group (Case 1) were -4,179, -4,512, and -4,343 ng/mL, respectively. In the low-dose group, Case 2 showed changes in ferritin levels at weeks 8, 16, and 24 of -2,974, -3,255, and -3,172 ng/mL, respectively (the study drug was discontinued on Day 71). In another participant (Case 3) in the low-dose group, the changes at weeks 8, 16, and 24 were +153, +153, and +284 ng/mL, respectively. Case 4, the changes in serum ferritin levels at weeks 8 and 16 were +7,753 and +129 ng/mL, respectively. The study drug was discontinued on Day 37. In the high-dose group, serum ferritin levels improved by week 8, and continued to improve thereafter.

The changes in the EQ-5D-5L scores at weeks 4, 8, 12, 16, 20, and 24 in the high-dose group (Case 1) were +0.85, +0.90, +0.90, +0.90, +0.90, and +0.78, respectively. Case 2 were +0.16, +0.16, +0.16, +0.16, +0.11, and +0.16. Case 3 were +0.21, +0.08, +0.16, +0.16, +0.16, and +0.08, respectively. Case 4, the changes in scores at weeks 4, 8, 12, and 16 were -0.05, +0.04, +0.18, and +0.12, respectively.

In the EQ-5D-5L VAS (where 100 represents the best possible state and 0 represents the worst possible state), the changes in the EQ-5D-5L VAS from baseline to each time point at weeks 4, 8, 12, 16, 20, and 24 in the high-dose group (Case 1) were +75, +79, +79, +80, +80, and +60, respectively. In the low-dose group, one participant (Case 2) showed the EQ-5D-5L VAS changes of +40, +40, +10, 0, +10, and +40, respectively. Another participant (Case 3) showed EQ-5D-5L VAS changes of +15, -25, +15, -5, -15, and -35, respectively. For a fourth participant (Case 4), the EQ-5D-5L VAS changed by -35, +5, +10, and +10 at weeks 4, 8, 12, and 16, respectively.

3.4. Safety

Adverse events (AEs) were observed in three of four participants (one in the high-dose group and two in the low-dose group), with a total of 11 events (four in the high-dose group and seven in the low-dose group). Table 3 lists the AEs. In the low-dose group, one participant (Case 3) experienced no AE during the 24-week period, including the 8-week period of the prior study. All AEs reported in this clinical study were determined to have no causal relationship with the study drug. One case of a serious AE (compression fracture) and one case of an AE leading to discontinuation of the study drug (drug eruption) were each reported in the same participant (Case 2), but both AEs were determined to have no causal relationship with the study drug. However, because the drug eruption was initially suspected to be a side effect of the study drug, administration of the study drug was suspended. As the suspension period exceeded 14 days, administration of the study drug was discontinued on Day 71. No deaths, AEs leading to death, or AEs leading to trial discontinuation were observed.

Table 3. Adverse events

Adverse event (preferred term)	n (%)	Study drug discontinuation	Association with study drug	Serious adverse event
High-dose group (*n* = 1)				
Conjunctival haemorrhage	1 (25%)	No	No	No
Hordeolum	1 (25%)	No	No	No
Influenza	1 (25%)	No	No	No
Purpura senile	1 (25%)	No	No	No
Low-dose group (*n* = 3)				
Conjunctivitis	1 (25%)	No	No	No
Cytomegalovirus infection	1 (25%)	No	No	No
Drug eruption	1 (25%)	Yes	No	No
Oral candidiasis	1 (25%)	No	No	No
Skin erosion	1 (25%)	No	No	No
Compression fracture	1 (25%)	No	No	Yes
White blood cell count decreased	1 (25%)	No	No	No

The data are *n*, %. The terminology is based on the Medical Dictionary for Regulatory Activities version 25.0.

4. Discussion

This study represents the first investigator-initiated Phase II clinical trial in the world to evaluate the efficacy and safety of 5-ALA-HCl/SFC in patients with GC-refractory, disease-active AOSD. Although enrollment was originally planned for 30 patients, recruitment did not progress as expected, and the trial was terminated early after the enrollment of four participants. Therefore, the results of this study should be regarded as pilot data, and definitive conclusions regarding the efficacy and safety of 5-ALA-HCl/SFC in AOSD cannot be drawn. Nevertheless, we believe that the findings provide useful preliminary information that may contribute to the design of future clinical studies.

Regarding efficacy, one participant (Case 1) in the high-dose group demonstrated improvements across multiple endpoints, including adapted ACR achievements, SFS scores, EQ-5D-5L scores, and serum ferritin levels. Among the three participants in the low-dose group, two (Case 2 and Case 4) discontinued study drug administration during the trial and subsequently switched to alternative treatments after week 12 and week 8, respectively. Case 3 continued the study drug through week 24, allowing a straightforward evaluation; however, no apparent improvement was observed in any efficacy indicators. These included composite measures (adapted ACR achievements, SFS scores), laboratory markers (ferritin, CRP, ESR), and patient-reported outcomes (EQ-5D-5L). On the other hand, Case 2 showed improvements in adapted ACR, SFS scores, ferritin, CRP, ESR, and EQ-5D-5L from baseline through week 8 while on the study drug, along with a reduction in prednisolone dose. Although the sample size is extremely small and no firm conclusions can be reached, these observations suggest that 5-ALA-HCl — particularly at a dose of 300 mg (with SFC 235.2 mg) per day — may have therapeutic potential in AOSD.

Elevated HO-1 levels have been reported in AOSD, and a correlation with ferritin levels has been established (*3,4*). HO-1 is a stress-responsive enzyme and a powerful anti-inflammatory and immune regulatory molecule (*18*). It is known to suppress inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-18, *etc.*) and induce anti-inflammatory cytokines such as IL-10 through the production of heme metabolites (CO, bilirubin) (*19*). It has been suggested that induction of HO-1 may mitigate cytokine storms and immune hyperactivity in AOSD. We focused on this point and considered that induction of HO-1 expression through 5-ALA/SFC administration could serve as a therapeutic target for AOSD. In preclinical studies, 5-ALA showed preventive and therapeutic effects in collagen-induced arthritis in DBA/1 mice. In addition, no significant effect on adaptive immune cells was observed, and 5-ALA mainly acted on macrophages and dendritic cells, which was reflected in a significant decrease in serum chemokines IP-10 and MIP-1β (*20*). 5-ALA/SFC suppressed systemic inflammation in a mouse model of AOSD, a systemic disease, and its severe form, macrophage activation syndrome, using C57BL/6N mice repeatedly administered CpG phosphorothioate oligodeoxynucleotides (*20*). Additionally, in patients with AOSD inadequate response to existing treatments (excluding severe cases), supplementation with 5-ALA enabled reduction of prednisolone dosage (*20,21*).

Regarding the treatment of AOSD, aside from GCs, biological agents targeting elevated cytokines such as IL-6 and IL-1β have been the mainstay. However, these agents have issues such as immunosuppression-related infections, the need for intravenous or subcutaneous injections, and high costs. In contrast, 5-ALA-HCl/SFC offers the advantages of being an oral treatment with a simple administration regimen and having fewer concerns regarding immunosuppression compared to GCs or biological agents.

The limitations of this study are as follows: Firstly, the sample size was limited to just four cases. While the primary endpoint was achieved by all participants, this may have been influenced by the small number of cases enrolled. The effect of concomitant GCs should also be considered. However, the participant in the high-dose group received a lower dose of concomitant GCs than the other three participants. Given the small cohort size and the inherent variability in AOSD, these findings should be interpreted with caution. Furthermore, participant background factors were heterogeneous, including age, weight, disease duration, prior medications, comorbidities and laboratory test results. The uneven distribution of cases between the treatment groups (one case in the high-dose group and three cases in the low-dose group) also made it difficult to interpret trends in efficacy, particularly with regard to potential dose-response relationships. Therefore, the results should be considered descriptive and exploratory, and no definitive conclusions can be drawn about the relative efficacy of different doses. Secondly, there is the issue of the validity of the assessment criteria. As there are no established clinical assessment tools for AOSD, the validity of this endpoint is uncertain. Nevertheless, the efficacy evaluation revealed similar trends in adjusted ACR, CRP, ESR and SFS values within the same participants. Furthermore, while 5-ALA-HCl/SFC is thought to exert anti-inflammatory effects by inducing HO-1, this study was unable to measure HO-1 in serum or peripheral blood mononuclear cells due to cost considerations. HO-1 should be included as a key biomarker in future studies. Thirdly, the observation period was limited to 24 weeks. A single serious adverse event, a compression fracture, was deemed to be unrelated to the study drug, and tolerability at 24 weeks was good. Nevertheless, two participants discontinued the study drug during the trial, which makes a longer-term evaluation of efficacy and safety desirable.

Despite early termination, a very small sample

size (n = 4), and a 16-week treatment period with assessments through Week 24, all participants met the primary endpoint (adapted ACR30 at Week 16); the high-dose participant demonstrated the most consistent improvements across secondary outcomes, and no treatment-related safety concerns were identified. These hypothesis-generating findings offer preliminary evidence that 5-ALA-HCl/SFC may provide clinical benefit in refractory AOSD. Larger, adequately powered randomized trials are warranted to confirm efficacy, clarify dose–response relationships, and further characterize safety.

5. Conclusion

This study provides preliminary efficacy signals (all participants achieved ACR30, with more significant improvements in the high-dose group) and safety evidence (no treatment-related adverse events) for 5-ALA-HCl/SFC in the treatment of GC-refractory AOSD. However, due to limitations such as small sample size and uneven group distribution, the efficacy and safety profiles of 5-ALA-HCl/SFC cannot be established. In the future, large-sample, randomized controlled trials are needed to verify the dose-response relationship and long-term efficacy, while incorporating HO-1 detection to improve mechanistic validation.

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