ALA-Porphyrin Science

Phase 2 randomized clinical trial of 5-Aminolevulinic acid plus sodium citrate chloride vs placebo for Covid-19 infected patients recovered with sequelae

Kyoko Imamura,*[a,b] Hidemitsu Sugihara[a,b] and Koichi Hirahata[c]

[a] *Social Cooperation Program of IT Healthcare, Graduate School of Pharmaceutica Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan. Fax: 03-5841-0281; Tel: 03-5841-0286; E-mail: kimamura@mol.f.u-tokyo.ac.jp*

[b] *Japanese Institute for Public Engagement, 1-21-23 Nagatsuta, Midori-ku, Yokohama, Japan, Tel: 080-3523-9261; E-mail: Info@ji4pe.tokyo*

[c] *Hirahata clinic, 1-24-6 Shibuya, Shibuya-ku, Tokyo, Japan, Tel: 03-3400-3288; E-mail: koichi@hirahata.com*

Summary.

Due to Covid-19 pandemic, development of safe and effective vaccines and medications has become accelerated. Public interests are also on surge and increasing number of food supplements and nutritional products are used, which alarms safe use of these products usually taken without supervision by healthcare professionals such as physicians and pharmacists.

5-Aminolevulinic acid with sodium ferrous chloride (5-ALA/SFC) has been on the market for users with fatigue, less of sleep, and difficulty in sugar control. Its safety profile is already long established, but it'd be important to see if it can be safely used for those patients infected by Covid-19 with sequelae.

In our study, continuous biometric monitoring by smartwatch and applications installed on smartphone are used by patients taking either placebo or 5-ALA/SFC for 28 days, in addition to routine clinical and laboratory check up prior to the screening and at the end of observation period. Despite the limited sample size, differences before and after use of 5-ALA/SFC were followed in both groups. During 28 days' administration, little changes were observed in placebo group whereas significant weekly changes were observed in 5-ALA/SFC group. Future study with larger sample size is expected to confirm the observation from this exploratory study.

Introduction

Recently health promotion has become much closer to people's daily life thanks to the advancement of monitoring devices and programs to help them identify their biomedical information [1, 2]. Easy measurement and visual presentation of the results are readily available at any moment and help them better manage their health continuously by setting alarms to keep up timely measurement and take any necessary actions such as taking pills or going to see their doctors. A variety of medical devices are on the market with or without regulatory approvals, and the programs delivered as applications for smart-phone users are also widely adopted. The data obtained can be integrated with their electronic health records [3]. As such, these devices have contributed to drastic change in

clinical research by inviting shift to remote monitoring and decentralized clinical trials, which could be of important option in the wake of Covid-19 [4].

Due to Covid-19 pandemic, healthcare resources such as hospital beds for patients and healthcare workers and equipment for treatment and prevention are severely constrained and even affected delivery of usual healthcare [5]. Public health measurements such as travel restriction, hands washing and keeping social distances are now fundamental part of our daily life, and the use of food supplement and nutritional products are on rise for those who feel uneasy to visit clinic in fear of getting infected. As these products are not controlled by healthcare professionals, their safety profile should be shared with the users.

5-Aminolevulinic acid with sodium ferrous chloride (5-ALA/SFC) has been marketed as supplements for those who care for their health in expectation of maintaining their activity level [6]. 5-ALA/SFC has also been known as inhibitory to SARS-CoV-2 infection in vitro [7], and examined in clinical trial with Covid-19 patients to seek its efficacy and safety in infection [8. In our study, we used 5-ALA/SFC for those who recovered from Covid-19 with sequelae primarily to seek its safety information in this population. We also measured biometric status of their health status using smart-watch and smart-phone to explore possibility of identifying any changes.

Experimentals （Methods）

Study Design

This single-center, double-blinded, placebo-controlled phase-2 randomized clinical trial was conducted from May 31, 2021, to Mar 31, 2022, at Tokyo Center Clinic and led by Dr Hiroki Nagashima. The trial protocol is available in jRCT database [9]. The trial compared 5-ALA/SFC with placebo in patients with Covid-19 sequelae, feeling with fatigue. To participate, the site required approval by the Certified Review Board. Written informed consent was required from all patients before registration. This study followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline [10].

Eligibility

Patients must have been within six months since infected by Covid-19, 20 to 75 years old inclusive at the time of informed consent. Their level of fatigue should be 40 mm or more when measured by Visual Analogue Scale (VAS). They are requested to use smart-watch and smart-phone. Patients allergic to 5-ALA/SFC, or ingredient in the study drug, or porphyrin should not be able to participate, as well as those who are photosensitive or taking drugs known to cause photosensitivity. They must have normal liver function test (AST and ALT being more than twice the upper normal limit), not serious anemia (hemoglobin level less than 10g/dl), and hypoxic (SpO2 less than 95%). Patients and their families are diagnosed as porphyria should be excluded. Those with past history of hemochromatosis or viral hepatitis, allergic to smart-watch band should also be excluded. They must not be participating other clinical studies. Regarding vaccination, patients who received

Covid-19 vaccines within six months of informed consent must be excluded from this study. Any patients as judged inappropriate by the investigator should not be included in this study.

Biometric Analysis

In addition to the usual physical examinations and a battery of lab tests, patients are asked to use smart-watch and smart-phone in the first week after screening visit, to get familiar with the devices and reporting method of their measurement. Garmin Vivosmart 4 [11] is used as smart-watch to measure level of sleep, and iPhone as smart-phone to which applications are installed such as EQ5D5L Japanese version [12], electronic questionnaire asking symptoms, and VAS asking level of fatigue (standardized by Japanese Society of Fatigue Science) by positioning "no fatigue" at level 0 and "worst fatigue" at level 100 on horizontal line on smart-phone display [13]. Patients are also provided pulse oximeter to measure and report their SpO2 every day. Pittsburgh sleep quality index Japanese version [14] is used before and after the test agent administration.

Randomization and Treatment

Patients were randomized in a 1:1 ratio to receive either 5-ALA/SFC or placebo. Randomization was stratified by sex (men vs women).

For patients randomized to 5-ALA/SFC group, three capsules of 50 mg 5-ALA and 29mg SFC were taken twice a day. For patients randomized to placebo group, matching number of placebo were given in the same manner. Disease assessments occurred at the start and end of treatment. After completion of treatment, patients were observed for further two weeks to evaluate safety of treatment.

End Points

The primary end point for the trial was the safety of the treatment, which was defined as the type and frequency of adverse events, diseases, and adverse drug reactions. Reported cases were categorized by frequency, seriousness, and expected or unexpected. Secondary endpoints included change in patient-reported level of fatigue by VAS; change in scores by Pittsburgh sleep quality index; change in sequelae reported at the start of treatment; change in quality of life measured by EQ5D5L; change in values of activity, body battery, and stress; sleep analysis as measured by smart-watch. Any other changes in lab test were also analyzed.

Statistical Analysis

Summary statistics were calculated on the number of patients, mean, standard deviation, median, interquartile range. Statistical significance was tested at 2-sided 0.05 level. 95% Confidence interval was calculated. For primary endpoint, frequency of occurrence of adverse events, diseases, and adverse drug reactions was analyzed in full analysis set. For secondary endpoints, Wilcoxon rank sum test was used for inter-group comparison. To compare weekly changes in fatigue VAS with

baseline assessment, for example, Wilcoxon signed-rank test was used. Also changes in values and ratio were summarized and presented in 95% confidence interval of mean was calculated. Other endpoints were analyzed in the same manner.

For analysis, aggregate, and table presentation, SAS ver 9.4 was used. For line listing, figures, and tables, Microsoft Excel and Microsoft PowerPoint were used.

Results and discussion

Patient Characteristics and Treatment

From May 31 to Sept 30, 2021, 46 patients were enrolled, of whom 42 patients were eligible for treatment. 21 patients were randomized to placebo group and 21 to 5-ALA/SCF group. After 2 patients withdrew from the study, the number of patients who completed 28 days' treatment was 21 in 5-ALA/SFC group and 19 in placebo group. Details of patient enrollment, eligibility, and treatment are provided in Figure 1, and baseline characteristics are described in Table 1. There was no urgent key-open, and data were fixed until all patients were assessed as valid for analysis.
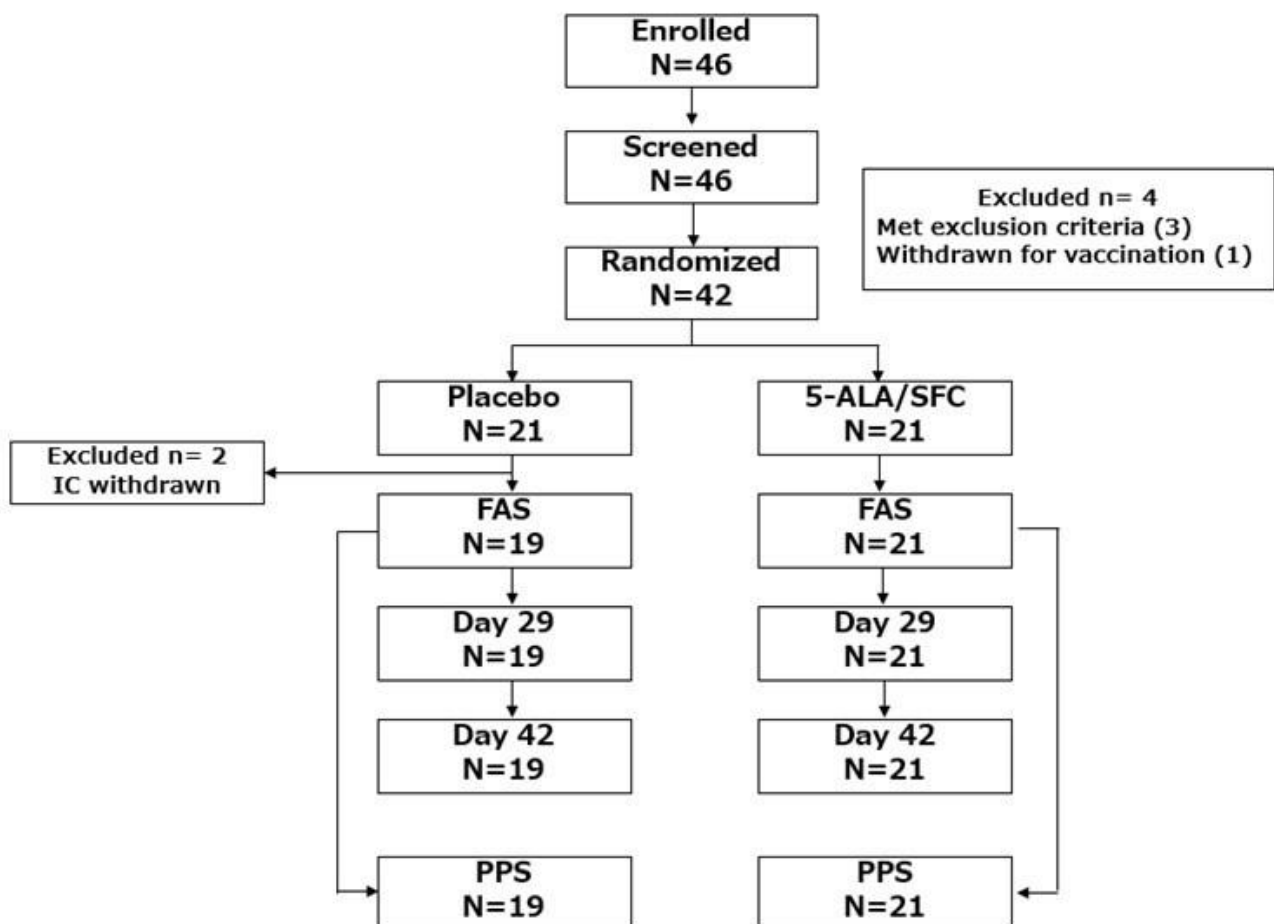
Figure 1. CONSORT Diagram

		Total	Placebo	5-ALA/SFC	P-value
Total	number	40	19	21	
Gender	Male	17	8	9	>0.999
	Female	23	11	12	
Age	Mean+/-SD	42.4 +/- 11.9	44.8 +/- 12.0	40.3 +/- 11.6	0.237
Symptom n (%)	Dyspnea	14 (35.0)	6 (31.6)	8 (38.1)	0.748
	Memory impairment	13 (32.5)	7 (36.8)	6 (28.6)	0.738
	Hair loss	10 (25.0)	4 (21.1)	6 (28.6)	0.721
	Chest pain	17 (42.5)	8 (42.1)	9 (42.9)	>0.999
	Loss of concentration	35 (87.5)	18 (94.7)	17(81.0)	0.345
	Joint pain	12 (30.0)	7 (36.8)	5 (23.8)	0.494
	Olfactory disorder	18 (45.0)	10 (52.6)	8 (38.1)	0.525
	Dysgeusia	14 (35.0)	9 (47.4)	5 (23.8)	0.186
	Cough	12 (30.0)	8 (42.1)	4 (19.0)	0.170
	Dryness	12 (30.0)	7 (36.8)	5 (23.8)	0.494
	Rhinitis	13 (32.5)	9 (47.4)	4 (19.0)	0.091
	Red eye	6 (15.0)	3 (15.8)	3 (14.3)	>0.999

Table 1. Baseline charateristics

Safety

Frequency of adverse events, diseases, and adverse drug reactions are tabulated in Table 2.

| Treatment	Symptom	Severity	Recovery	Seriousness	Causality
5LA/SFC	Diarrhea	1	Recovered	No	Denied
	Wheal	1	Recovered	No	Denied
5LA/SFC	Acute gastroenteritis	1	Recovered	No	Denied
Placebo	Left foot arthralgia	1	Recovered	No	Denied

Table 2. Adverse events observed

There was no statistically significant differences between groups in both full analysis set and per protocol set, Three adverse events were found in 2 patients of 5-ALA/SFC group (acute gastroenteritis 1, diarrhea 1, wheal 1). Whereas one adverse event in one patient of placebo group (arthralgia of left foot).

Efficacy

Patients were asked to score their level of fatigue every day to standardize their skill of assessment. After this exercise for 7 days, they started to receive treatment. The VAS values of each group over 4 weeks' treatment are shown in Figure 2. Compared to VAS value at Day 0 as baseline score, no

statistical difference was found between the two groups. If looked at intra-group changes, however, 5-ALA/SFC group showed significant differences from baseline in consecutive weeks of week 3 and week 4.

Patient's quality of life was measured using EQ5D5L, in which statistical significance (p=0.033) was observed in the level of anxiety/depression in 5-ALA/SFC group during 4 weeks' treatment whereas no significant change in placebo group (Figure 3). Other measures by EQ5D5L (mobility, self-care, usual activities, pain/discomfort) were all statistically significant during 4 week's treatment, although not significant between the groups.
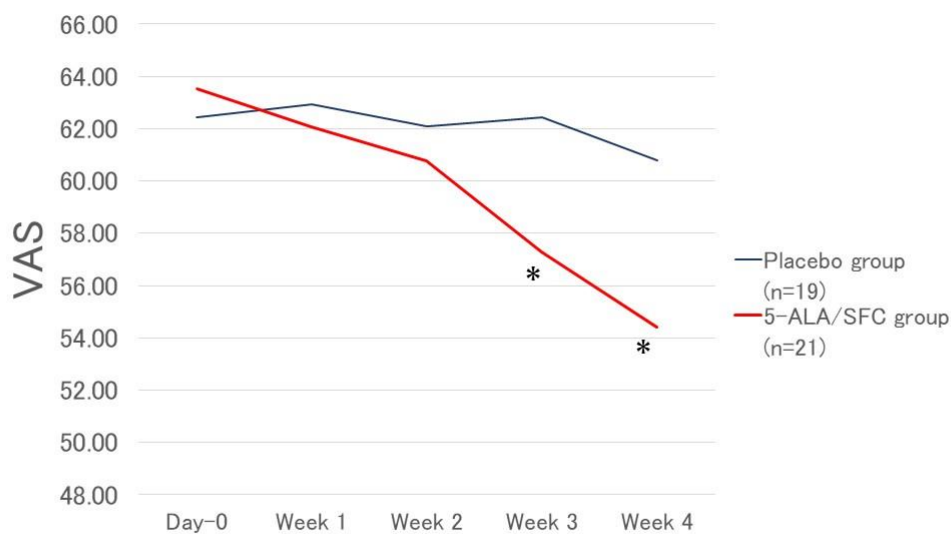
Figure 2. Level of fatigue

NS: inter-group (Wilcoxon rank sum test), *: p<0.05 (Wilcoxon signed-rank test)
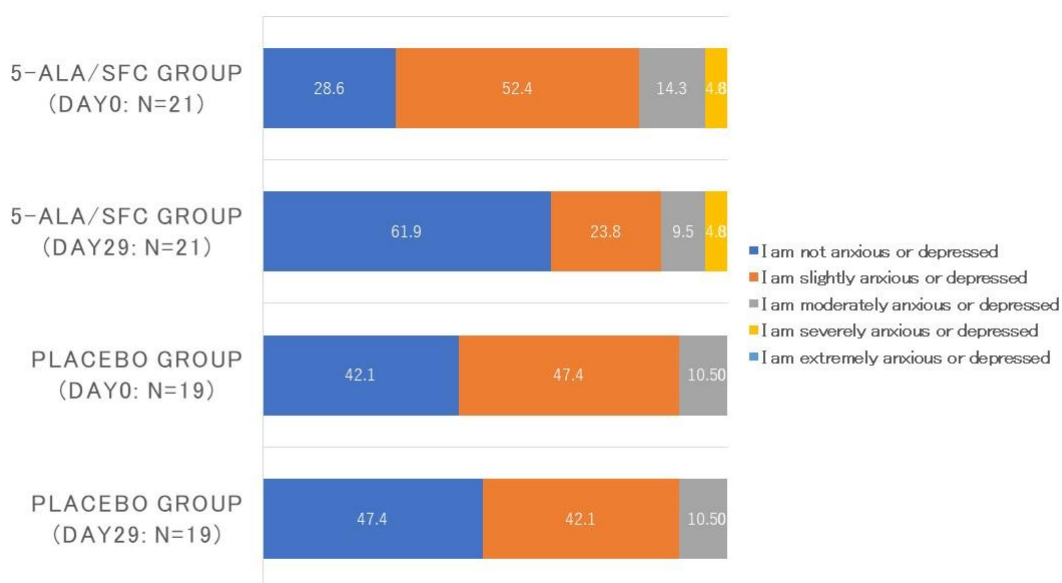
Figure 3. Anxiety/Depression

Day 0 vs Day 29: 5-ALA/SFC group: p<0.05、Placebo group: NS (Wilcoxon signed-rank test)

Limitations

As discussed, the main limitation of our study is that due to the arrival of vaccines in Japanese marketplace and the national policy to get as many vaccinated as soon as possible, recruiting time was limited (in our study, one patient stopped study participation to get vaccinated) to maintain study eligibility to achieve homogeneous study population. Another limitation is a short of funding resulted in call for participation limited in Tokyo area. When decentralized clinical trial becomes feasible in Japan, this limitation may be overcome by calling multiple sites to join the study so that patients can participate wherever they are.

<u>**Conclusion**</u>

5-ALA/SFC was safely used in the examined dose by patients recovered from Covid-19 with sequelae. Despite limited number of target participants, statistically significant change was observed in the level of fatigue as well as anxiety/depression. As the number of infected patients grows rapidly with Omicron variant, more number of patients are left with sequelae and further study is needed with larger sample size and a range of dosing levels.

References

1 B. Reeder, A. David, Journal of Biomedical Informatics, 2016, 63, 269

2 JC. Moses, S. Adibi, SMS. Islam, N. Wickramasinghe, L.Nguyen, Healthcare, 2021, 9, 889

3 Alpert JM, Kota NSP, Ranka S, Mendoza TV, Solberg LM, Rashidi P, Manini TM, JMIR Hum Factors 2020;7(4):e19769

4 MA. Banks, Proceedings of Natural Academy of Sciences, 2021, 118, 47, e2119097118

5 V. Kumar, H. Alshazly, SA. Idris, S. Bourouis, Sustainability, 2021, 13, 13642

6 K. Takahashi, Journal of Arid Land Studies, 2018, 28-2, 66-72

7 Y. Sakurai, MMN. Tun, Y. Kurosaki, T. Sakura, DK. Inaoka, K. Fujine, K. Kita, K. Morita, J. Yasuda, Biochemical and Biophysical Research Communications, 2021, 545, 203

8 https://rctportal.niph.go.jp/detail/jr?trial_id=jRCTs031190094

9 https://jrct.niph.go.jp/latest-detail/jRCTs031210123

10 http://www.consort-statement.org/

11 https://www.garmin.co.jp/products/intosports/vivosmart-4-black-r/

12 S. Ikeda, T. Shiroiwa, A. Igarashi, S. Noto, T. Fukuda, S. Saito, K.Shimozuma, J. Natl. Inst. Public Health, 2015, 1, 64

13 https://www.hirougakkai.com/

14 Y. Doi, M. Minowa, M. Okawa, M. Uchiyama, Japanese Journal of Psychiatry Treatment, 1998, 13 (6): 755