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Applied nutritional investigation

5-aminolevulinic acid, a precursor of heme, reduces both fasting and postprandial glucose levels in mildly hyperglycemic subjects

Fumiko Higashikawa Ph.D. [a,b], Masafumi Noda Ph.D. [a,b], Tomokazu Awaya M.D., Ph.D. [c], Tohru Tanaka Ph.D. [d], Masanori Sugiyama Ph.D. [a,b,*]

[a] Project Research Center for Clinical Trial and Preventive Medicine, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
[b] Department of Molecular Microbiology and Biotechnology, Graduate School of Biomedical Sciences and Health, Hiroshima University, Hiroshima, Japan
[c] Clinical Research Center, Hiroshima University Hospital, Hiroshima, Japan
[d] SBI Pharmaceuticals Co., Ltd., Tokyo, Japan

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ABSTRACT

Objective: The aim of this study was to evaluate the combined effects of 5-aminolevulinic acid phosphate (ALA-P) and iron on the glycemic index in mildly hyperglycemic adults.
Methods: This double-blind, randomized placebo-controlled trial comprised 212 subjects (ages 35–70 y, fasting plasma glucose 105–125 mg/dL or hemoglobin (Hb)A_{1c} 6.1%–7.1%). These participants were randomly assigned to four groups receiving either one of three doses of ALA-P and iron as sodium ferrous citrate (5 mg and 0.6 mg, 5 mg and 1.8 mg, or 15 mg and 1.8 mg, respectively) or a placebo, administered orally once a day over a 12-wk period.
Results: Fifteen mg ALA-P plus 1.8 mg iron decreased the fasting plasma glucose level (2.32 mg/dL, 95% confidence interval [CI], 0.24–4.42, $P = 0.029$), serum glycoalbumin (0.22%, 95% CI, 0.02–0.42; $P = 0.031$), and 2h-oral glucose tolerance test levels (14.2 mg/dL, 95% CI, 1.8–26.6; $P = 0.025$) more than the placebo. However, the levels of HbA_{1c}, fasting insulin, serum 1,5-anhydro-D-glucitol, and Homeostasis Model of Assessment-Insulin Resistance showed no appreciable changes. The participant numbers with impaired glucose tolerance and impaired fasting glucose decreased in the highest dosage group of ALA-P plus iron compared with the placebo group.
Conclusion: An oral intake of ALA would be a novel approach to prevent type 2 diabetes mellitus.

Introduction

Diabetes mellitus (DM) is rapidly increasing worldwide with a prevalence of 6.4% (285 million adults) in 2010. It is estimated that it will increase to 7.7% (439 million adults) by 2030 [1]. Several studies have indicated that individuals with prediabetes also may suffer with diabetic complications, such as retinopathy and peripheral neuropathy [2,3]. According to the current treatment guidelines, borderline diabetes becomes scarcely a target of the medical treatment of the medicines for diabetes treatment. Early care is important for individuals with prediabetes to prevent diabetes-related complications and/or the onset of DM.

In the present clinical trial, we evaluated 5-aminolevulinic acid (ALA), which is dissimilar to any of current antidiabetic agents in clinical use. ALA is endogenous to both animals and plants and is the first compound produced in the heme biosynthetic pathway. The responsible enzyme is ALA synthase, which is rate limiting in this pathway. Iron is ultimately incorporated into protoporphyrin to form heme in mitochondria. Heme is a major component of hemoglobin, and of other hemoproteins including myoglobin and cytochrome. Cytochromes play an important role in the electron transport chain in mitochondria, and cytochrome P450s function as metabolic enzymes involved in the oxidation and detoxification of many xenobiotics and endogenous compounds, and in fatty acid desaturation. Hence, as the precursor of heme, ALA is an essential molecule in human and other vertebrates, and may be associated with various metabolic disorders. Indeed, it has been reported that the administration of ALA together with iron stimulates murine hair growth [4].

It has been shown that mitochondrial dysfunction is associated with insulin resistance and type 2 diabetes mellitus (T2DM) [5]. Whether the impaired mitochondrial function is a cause or consequence of insulin resistance is not clear yet. However, the

FH and MS designed the research, and had primary responsibility for the final content. FH, MN, and TA conducted the research. MN and TA were responsible for the data collection and participants. TT provided the capsules used in the study. FH analyzed the data, and wrote the paper.

* Corresponding author. Tel.: +81 (82) 257 5280; fax: +81 (82) 257 5284.
E-mail address: sugi@hiroshima-u.ac.jp (M. Sugiyama).

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defective insulin signaling may promote mitochondrial dysfunction because the mitochondrial function is lower in patients with genetically defective insulin receptors than in healthy controls [6]. Porphyria cutanea tarda is caused by abnormalities in heme biosynthesis and often is accompanied by DM [7]. Moreover, the activity of ALA dehydratase is found to be reduced in a streptozotocin-induced diabetic rat model [8–10]. We thus speculated that the heme biosynthetic pathway may be associated with glucose metabolism, and that mitochondrial activity may be enhanced by the supplementation of ALA whereby the heme biosynthesis is augmented. In the present study, we tried to substantiate the hypothesis by the combined oral administration of 5-aminolevulinic acid phosphate (ALA-P) and iron in a human cohort.

Materials and methods

Participants

Healthy volunteers were recruited from the local community in Hiroshima, Japan through a series of advertisements. The inclusion criteria were as follows: 1) healthy men or women between the ages of 35 and 70 y; and 2) a fasting plasma glucose (FPG) level of 105 mg/dL to 125 mg/dL or a hemoglobin (Hb)A_{1c} of 6.1% to 7.1%. The following exclusion criteria were applied: 1) taking medication for diabetes 2) a body mass index (BMI) <18 kg/m^2 or >30 kg/m^2; 3) pregnant or breastfeeding; 4) renal or hepatic dysfunction; 5) heart disease; 6) history of porphyria, hemochromatosis, or viral hepatitis; 7) functional food intake that may affect plasma glucose level; and 8) participation in any other clinical trial within 90 d of the commencement of this study. The study protocol was approved by the Ethics Committee of Hiroshima University and performed in accordance with the guidelines of the Helsinki Declaration. All participants provided written informed consent before the start of the trial.

Study design

The current trial was a double-blind, randomized, placebo-controlled, parallel-group study conducted at Hiroshima University Hospital, Hiroshima, Japan, from May 2010 to December 2010. The purified ALA-P used in this study is a fermentation product of the photosynthetic bacterium *Rhodobacter sphaeroides*. The capsules used to administer the ALA-P and iron in the form of sodium ferrous citrate were provided by SBI Pharmaceuticals Co., Ltd., Tokyo, Japan.

The 212 eligible participants assessed in this trial were enrolled and stratified according to sex and the HbA_{1c} level (of $\geq$6.3% or less) at baseline by an investigator, and assigned to one of four treatment groups (placebo; 5 mg ALA-P plus 0.6 mg iron; 5 mg ALA-P plus 1.8 mg iron; or 15 mg ALA-P plus 1.8 mg iron) by means of blocked randomization with a block size of 4 and an allocation ratio of 1:1:1:1 using computer-generated random numbers. Randomization assignments were carried out by non-clinical staff who had no subsequent involvement with the trial. The volunteers and outcome assessors were kept blinded to the allocation. The participants were instructed to maintain their ordinary dietary habits during the study period and to take three capsules per day after dinner for 12 wk. They were also asked not to donate blood during the trial. Clinical visits were scheduled every 4 wk at Hiroshima University Hospital, at which time physical examinations, hematologic assessments, serum biochemical measurements, and urinalyses were performed. The oral glucose tolerance test (OGTT), in addition to the fasting insulin, serum adiponectin, leptin, and resistin level tests, was performed at weeks 0 and 12. Blood samples were taken after an overnight fast (of at least 9 h). The body fat percentage was measured using a body composition analyzer (BC-118E; Tanita, Tokyo, Japan). HbA_{1c} values were converted from Japan Diabetes Society values to those of the National Glycohemoglobin Standardization Program by adding 0.4% [11]. Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) was calculated as fasting insulin (μU/mL) $\times$ fasting glucose (mg/dL)/405. The participants were provided daily and dated record forms throughout the study period, including 2 wk of run-in period and 4 wk of follow-up period, to make a note of their capsule consumption and health conditions. Adverse events (AEs) that newly emerged or worsened after the intervention were assessed as grade 1 (mild), 2 (moderate), or 3 (severe), as according to the Common Terminology Criteria for Adverse Events version 3.0. Compliance with the treatment regimen was assessed using participants' daily records.

Statistical analysis

The sample size was calculated at 45 participants per group with 90% power and a significance level of 0.05 using the two-sided Student's *t* test to detect a 5% difference with an estimated SD of 8% between groups. The baseline characteristics were compared among groups by one-way analysis of variance for continuous variables. The Fisher's exact test was used for all categorical variables to assess differences among the four study groups. Data analysis was carried out as an intention to treat, and the multiple imputation method was applied to missing data. The mean values during the intake (weeks 4, 8, and 12) were used to compare with each baseline for main outcomes. A general linear model of analysis of covariance (ANCOVA) followed by a post hoc multiple *t* test between all paired groups were applied to changes from the baseline of the main study end points including the FPG, glycoalbumin, HbA_{1c}, 2-h glucose concentration after the OGTT (2h-OGTT), fasting insulin, HOMA-IR, and 1,5-anhydro-D-glucitol (1,5-AG) levels, by using each baseline value and the participant's sex as covariates. The 2h-OGTT changes were assessed by subgrouping participants based on baseline values, all participants, participants with $\geq$140 mg/dL, or with $\geq$200 mg/dL, and were calculated as the mean change from the baseline adjusted by ANCOVA as described previously. The Fisher's exact test with a Bonferroni correction was applied to the change in subject number with both an 2h-OGTT $\geq$140 mg/dL and an FPG $\geq$110 mg/dL. Statistical analyses were performed using SPSS (version 17.0, SPSS Japan, Inc.), data are expressed as mean $\pm$ SD (for tables) or SEM (for figure), and $P < 0.05$ was considered significant.

Results

Figure 1 shows the profile of the current study trial. Of the 701 individuals who expressed an interest in participating in the

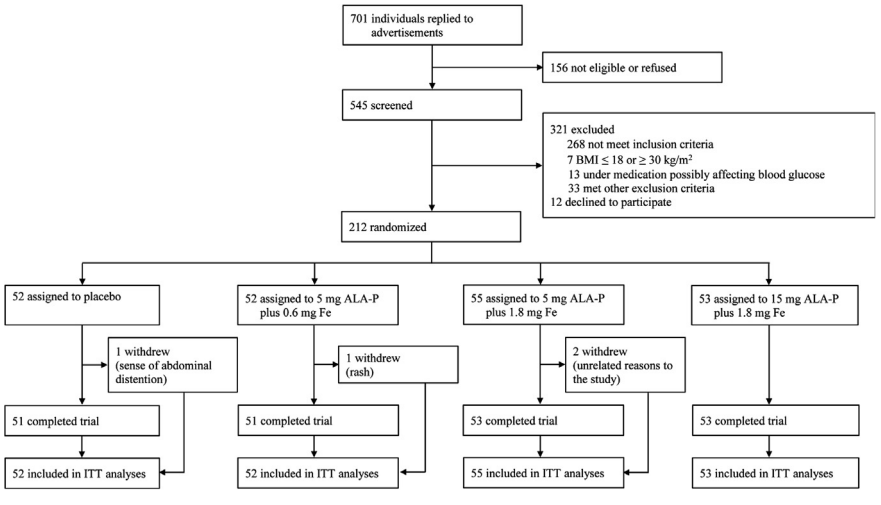

Fig. 1. Trial profile.