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Five-aminolevulinic acid: New approach for congenital sideroblastic anemia

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Congenital sideroblastic anemia (CSA) is a rare disease caused by mutations of various genes involved in heme biosynthesis, iron–sulfur cluster biogenesis or mitochondrial protein synthesis, resulting in formation of ring sideroblast cells. In normal erythroid cells, the heme biosynthetic pathway begins with a reaction to yield 5-aminolevulinic acid (ALA) in the presence of 5-aminolevulinate synthase 2 (ALAS2), and in the final step iron is incorporated into protoporphyrin IX (PPIX; Fig. 1a).[1] The main cause of this disease is a mutation in *ALAS2* in the Japanese population. With the exception of pyridoxine-responsive patients, therapy for CSA has been mainly supportive. Given that ALA supplementation, theoretically, could be an effective therapeutic strategy to restore heme synthesis in CSA caused by ALAS2 defects,[2] we investigated the safety and efficacy of ALA treatment. This study was approved by the Institutional Review Board of Matsushita Memorial Hospital.

A Japanese boy was noted to have anemia (hemoglobin [Hb], 7.0 g/dL) at the age of 2 years. The definitive diagnosis were not obtained at that time, but hemoglobin concentration gradually improved to 10.0 g/dL without any treatment. He was referred to again at the age of 19 years because of general fatigue. Hb, mean corpuscular volume and mean Hb concentration were 7.8 g/dL, 73.9 fL and 22.2 pg, respectively, and other blood cell counts were normal. Bone marrow aspiration indicated typical ring sideroblasts without myelodysplastic morphology. No causative mutations were found in *ALAS2*, *SLC25A38*, *GLRX5*, *ABCB7*, *PUS1* or *SLC19A2*. The anemia gradually progressed without any response to pyridoxine. Hepcidin and ferrochelatase activity were normal. Finally, a deletion of 35 bp was identified in the first intron of *ALAS2*. He was diagnosed with CSA due to loss of function of an erythroid-specific enhancer for *ALAS2*.[3] Because of the necessity for blood transfusion every couple of months, ALA was initiated after providing written informed consent. ALA-phosphate supplemented with iron 1.8 mg in the form of sodium ferrous citrate (kindly provided free of charge by SBI Pharmaceuticals, Tokyo, Japan) was progressively increased up to 315 mg (180 mg as ALA) twice a day before meals, and the final dosing was well tolerated with no side-effects (i.e. photosensitivity or gastrointestinal disturbance). After 16 weeks, however, no improvement was seen in clinical laboratory data (Table S1).

Blood ALA and its metabolites were measured at 9 time points during the 8 h after the first ALA dose, according to Ota *et al.*[4] in the setting of ALA 180 mg twice per day. ALA and PPIX peaked at 19.0 μmol/L at 2 h and at 0.056 μmol/L at 4.5 h after ALA-phosphate intake, respectively (Fig. 1b).

The patient was given a dose equal to approximately half the amount of endogenous synthesis (approx. 700 mg) in a healthy adult by ubiquitously expressed ALAS1 and erythroid-specific ALAS2 daily, resulting in an ALA blood concentration at the effective level (>10 μmol/L; defined according to *in vitro* experiments[2]). The increased levels of not only ALA but also PPIX accruing from exogenous ALA-phosphate suggested that a certain level of heme was synthesized in the patient. Given that exogenous ALA in plasma is likely to be carried ubiquitously into systemic cells by SLC36A1, a

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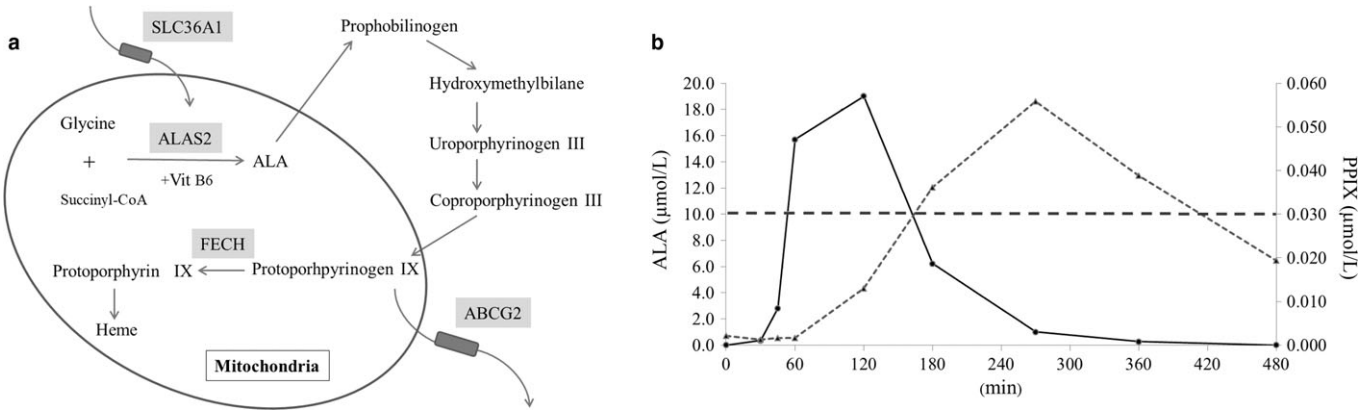

Fig. 1 (a) Heme biosynthesis. In normal erythroid cells, the heme biosynthetic pathway begins by condensing glycine with succinyl-coenzyme A (succinyl-CoA) to yield 5-aminolevulinic acid (ALA). The reaction requires pyridoxine as a cofactor to stimulate the enzymatic activity of 5-aminolevulinate synthase 2 (ALAS2). Heme synthesis progresses successively to protoporphyrin IX (PPIX), and in the final step of heme synthesis, ferrochelatase (FECH) is used to incorporate iron into PPIX. Exogenous ALA in plasma is likely to be carried ubiquitously into systemic cells by SLC36A1, a proton-coupled amino acid transporter. ABCG2, adenosine triphosphate (ATP)-binding cassette (ABC) transporter. (b) Change in (—) plasma 5-aminolevulinic acid (ALA) and (- - -) protoporphyrin IX (PPIX) with time after oral ALA-phosphate 315 mg (180 mg as ALA) twice per day in a congenital sideroblastic anemia patient. The measurements were done after the first dose of the day. ALA blood concentration reached an effective level (>10 μmol/L; defined in *in vitro* experiments using K562, an erythroid leukemia cell line[2]), for approximately 2 h per day. The calculated area under the ALA and the PPIX curves through 0–24 h was 39.4 and 0.39 μmol-h/L in the present case, respectively.

proton-coupled amino acid transporter,[5] ALA appeared, theoretically, to be delivered into the bone marrow cells, according to the cell number ratio for the total systemic cells. Given the lack of elevated Hb, it is presumed that exogenous ALA did not allow for significant conversion to heme in the erythroid cells. Any further increase of ALA dose, however, should be carefully considered because of the risk of photosensitivity. For these reasons, development of a novel drug delivery system to deliver ALA to the bone marrow is warranted for the treatment of CSA.

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Disclosure

The authors declare no conflict of interest.

Author contributions

H.I. and H.H. designed and wrote the study; H.I., T.I., A.M., T.F. and H.H. collected and analyzed the data. All authors read and approved the final manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Change in parameters after ALA intake.