Successful treatment of a Diamond-Blackfan anemia patient with amino acid leucine

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Diamond-Blackfan anemia (DBA; OMIM:105650) attracts much attention, because symptoms are associated with mutations in RPS19[1] and RPS24[2] in 25% and 2% of DBA patients, respectively, indicating a possible relationship between the ribosomal function, translation levels and erythropoiesis. Indeed, in our recent study we showed that translational efficiency was lowered in most DBA patients, and leucine was tested as a potential modulator of protein synthesis with promising results.[3] We therefore decided to test the effects of leucine in DBA patients.

For leucine therapy, we selected the patient with lowest levels of translation (25% of control basal translation; patient CZ23 in ref.[3]) and the best *in vitro* response to leucine (translation increased by >100%). The patient, now a 7-year old girl, was born from the first uncomplicated pregnancy. The diagnosis of DBA was confirmed at the age of 6 months. The patient has short stature and bilateral vesicoureteral reflux. No RPS19 mutation was found. Repeated courses of steroids including large doses[4] elicited no effect, and the girl entered a regular transfusion program. Iron chelation therapy was initiated at the age of 4 years, after liver hemosiderosis was observed. The search for a bone marrow donor has been unsuccessful.

Before the start of leucine therapy, leucine absorption tests were performed using 500mg and 1 500mg of leucine in one dose (the interval between tests was one month). The highest serum levels of leucine were observed two hours after leucine administration (Figure 1), while serum levels of other amino acids remained unchanged. A dose of 500mg of L-leucine has been then administered orally twice a day in the form of a capsule prepared by hospital pharmacy. The dose was based on the leucine absorption results (all leucine serum levels remained in normal range), and the leucine content in sport dietary protein supplements reduced according to the patient's body surface area.

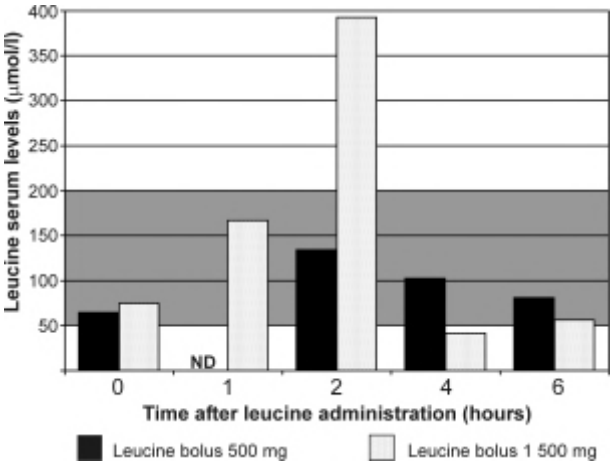
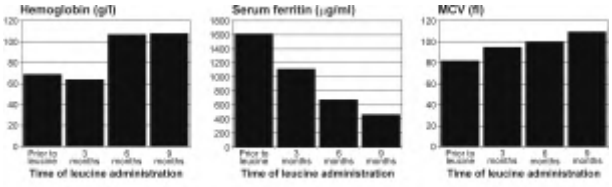


Figure 1. Kinetics of leucine absorption. Prior to the start of leucine therapy, two leucine absorption tests were performed after the oral administration of 500 mg or 1 500 mg of leucine in one dose. In both cases, the highest leucine serum levels were observed two hours after administration. Shaded area represents normal values of serum leucine levels in age-matched controls. ND: not done.



Parametr	Prior to leucine	3 months	6 months	9 months
Hemoglobin (g/l)	69	64	107	108
MCV (fl)	82	94.8	100	109.6
Reticulocytes (%)	0.002	0.003		0.019
Leukocytes (x10⁹/l)	4.2	4.89	3.71	4.9
Thrombocytes (x10⁹/l)	84	72	89	105
Serum ferritin (μg/ml)	1619	1117	681	469
ALT (μkat/l)	1.6	0.9		0.38
EPO (U/l)	1160			296
HbF (%)	0.7			5.6
BFU-E (per 10⁵ BM cells)	33			79.3
Erythropoiesis in BM (%)	3.2			26

Figure 2. Patient's characteristics during leucine therapy.

At the time of the start of leucine therapy, the child suffered from a lack of appetite and showed poor weight gain. After 3 weeks of leucine supplementation, her mother reported a noticeable increase in appetite and weight gain. Over a period of 6 months, a gradual improvement in reticulocyte count, hemoglobin level and reduction of serum ferritin level were observed; the patient became transfusion independent, and is currently still in remission (>5 months). At present, hemoglobin level is 90-105 g/l, MCV increased from 82 fl to clear macrocytosis (109.6 fl); HbF levels increased to 5.6% from a normal value of 0.7% at the time of transfusion dependency; BFU-E colonies from bone marrow increased in number and were better hemoglobinized (Figure 2).

Though we cannot exclude the possibility of spontaneous remission, our previous *in vitro* results favor the leucine-effect explanation.[3] Moreover, three other DBA patients that have recently started leucine therapy show increased appetite, growth and well-being, as it has already been described in patients with chronic diseases and in chicks,[5,6] supporting the hypothesis that the beneficial effect could be attributed to leucine alone. There are several reports describing the important role of the amino acid leucine in regulating protein synthesis by acting as a nutrient signal. Though it is not fully understood, it involves the mTOR pathway.[7] Via this pathway, leucine boosts translation by enhancing the activation of translation initiation factors that regulate mRNA binding to the ribosomal complex, and by a specific up-regulation of ribosome biosynthesis through the ribosomal protein S6 kinase.[8]

The administration of leucine thus offers the possibility of erythropoiesis stimulation without any of the well-known adverse effects of standard DBA treatment. However, these encouraging findings raise a number of questions related not only to DBA treatment (dose, regime, etc.), but also to general aspects of erythropoiesis. To answer these questions, studies involving more DBA patients are clearly required.

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