Haematological effects of oral administration of bitopertin, a glycine transport inhibitor, in patients with non-transfusion-dependent β-thalassaemia

Bitopertin is a small molecule selective inhibitor of glycine transporter 1 (GlyT1), initially developed to increase brain extracellular levels of glycine in the vicinity of neuronal *N*-methyl-D-aspartate receptors for the treatment of schizophrenia. GlyT1, the pharmacological target of bitopertin, is also present as a transmembrane transporter in erythroid cells[1] and accounts for 50–55% of glycine uptake in human red blood cells (RBCs).[2,3] Erythroid GlyT1 inhibition by bitopertin leads to reduced intracellular glycine availability, interfering with the first step of haem synthesis, in which 5-aminolevulinate synthase catalyses the condensation reaction between glycine and succinyl-coenzyme A, forming 5-aminolevulinic acid.[1]

β-Thalassaemia is a haemolytic anaemia arising from different genetic alterations of the β-globin gene (haemoglobin subunit beta), resulting in abolished or significantly reduced polypeptide β-globin chain synthesis and α-globin chain accumulation. Membrane damage due to unpaired α-globin chain deposition with an excess of free haem and iron contributes to ineffective erythropoiesis and decreased erythrocyte survival in the circulation. A reduced availability of haem and iron in β-thalassaemia may have beneficial effects in reducing ineffective erythropoiesis and improving RBC survival.[4,5] In a β-thalassaemia mouse model, oral administration of bitopertin resulted in reduced anaemia and haemolysis, enhanced *in vivo* survival of erythrocytes, and diminished ineffective erythropoiesis.[6] Markers of cellular damage induced by reactive oxygen species (ROS) were also substantially improved.

This report examines the haematological effects of oral bitopertin in patients with non-transfusion-dependent (NTD) β-thalassaemia. A summary of the clinical study protocol and data-sharing statement are provided in the supplementary online material (see Supplementary Material and Methods). Twelve patients were enrolled, five male (41·7%) and seven female (58·3%), with a mean (± standard deviation [SD]) age of 32·8 years (±9·7). Five patients (41·7%) were Asian and seven (58·3%) were Caucasian. Relevant clinical characteristics for all patients are presented in Table SI. Patients received oral bitopertin 30, 60 and then 90 mg/day during a six-week intrapatient dose escalation phase, followed by ≤10 weeks at the target dose of 90 mg. Figure S1 displays the length of treatment and bitopertin dosing. Six patients (50%) completed the initial 16-week treatment. Reasons for treatment discontinuation were: lack of efficacy (one patient, 8·3%), physician decision (one patient, 8·3%), and study termination by the sponsor (four patients, 33·3%).

Individual values for haemoglobin (Hb) during the trial are presented in Fig 1. The first eight patients assessed at an eight-week preliminary efficacy analysis showed a mean (±SD) total Hb reduction of −4·42 g/l (±6·85 g/l; Table I). Other selected biomarkers of disease activity, presented in Table I, did not show clinically meaningful improvement. The lack of improvement, or the actual decrease, in Hb values at the target dose of 90 mg was attributed to excessive inhibition of haem biosynthesis and/or globin chain production. Consequently, the target dose of bitopertin was decreased from 90 mg to 30 mg daily. Overall, the preliminary efficacy analysis led to treatment discontinuation in three patients and a two-week treatment interruption in four additional patients. Treatment was re-initiated at 30 mg for patients who interrupted treatment, and all newly enrolled patients remained on the starting dose of 30 mg/day throughout treatment (Figure S1). A subsequent analysis from 11 patients at Week 9 showed a total Hb value reduction of −3·4 g/l (±7·6) compared with baseline (Fig 1). Therefore, the study was terminated and the extension period cancelled.

Eight patients (66·7%) had adverse events (AEs) considered related to the study drug, and one patient experienced three serious AEs of Grade 3. The most frequent AEs were dizziness (41·7%), headache (25%) and decreased Hb (25%; Table SII).

Despite strong preclinical evidence for a potential therapeutic role of glycine restriction in a mouse model of β-thalassaemia,[6] the present study of oral bitopertin in patients with NTD β-thalassaemia failed to show clinically significant improvements in haematological and chemical biomarkers of disease activity. A therapeutic effect for bitopertin would have required a significant increase in RBC count in conjunction with either no change or a comparatively smaller reduction in mean corpuscular haemoglobin (MCH), as seen in the mouse β-thalassaemia model. However, in most patients, the decrease in MCH was greater in relative magnitude than the increase in RBC count, resulting in diminished total Hb.

The lack of therapeutic efficacy for bitopertin in human NTD β-thalassaemia challenges some of the assumptions

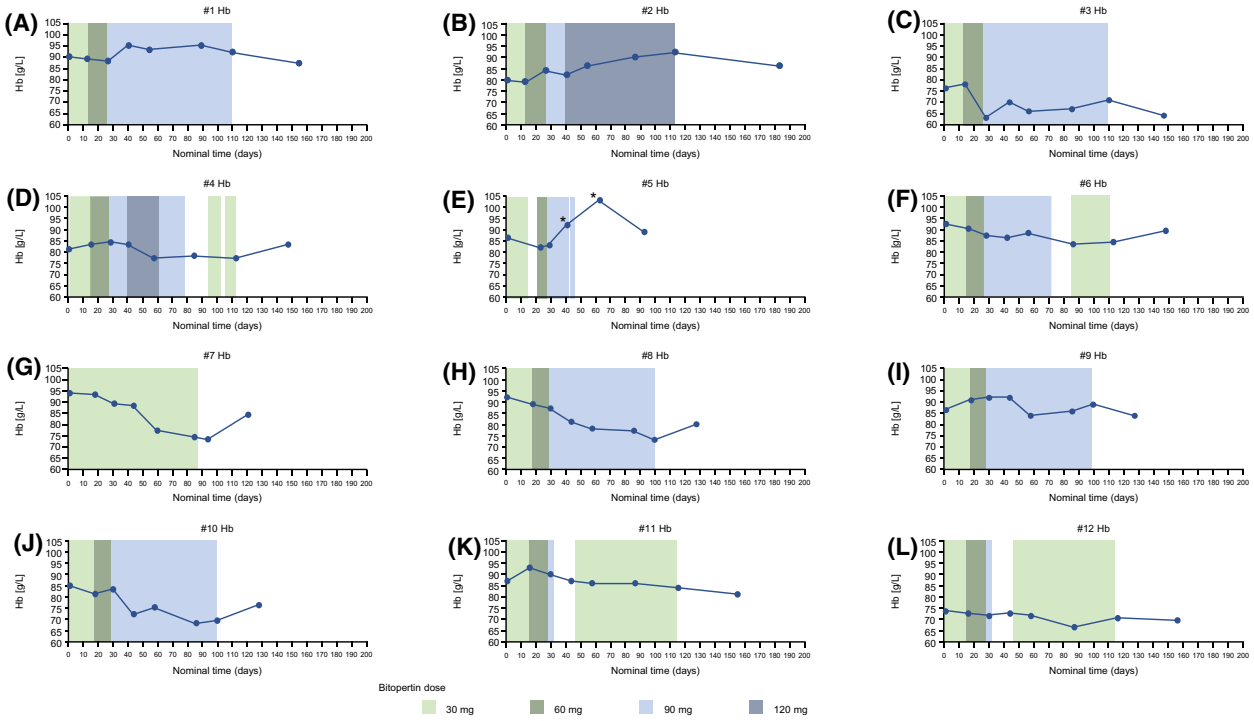

Fig 1. Individual haemoglobin (Hb) changes during the trial for patients with β-thalassaemia. (A–L) Hb levels in individual patients and treatment periods with bitopertin doses of 30, 60 and 90 mg/day. Patients #2 and #4 were also treated with 120 mg/day of bitopertin as depicted. White spaces are either treatment interruptions or follow-up periods. Dose levels depicted refer to daily dose. *Denotes visit occurring after the patient received a transfusion. [Colour figure can be viewed at wileyonlinelibrary.com]

Table I. Effect of bitopertin administration on relevant biomarkers in patients with NTD β-thalassaemia.

Parameter	Baseline mean (SD)	Eight-week mean (SD)	Mean absolute change from baseline (SD)	Mean % change from baseline (SD)
Hb (g/l)	85·4 (6·0)	81·0 (8·7)	−4·42 (6·85)	−5·15 (8·10)
MCH (pg)	20·47 (3·09)	18·46 (3·11)	−2·01 (2·55)	−9·26 (12·86)
Retic-Hb (pg)	22·05 (2·33)	20·25 (1·59)	−1·8 (1·42)	−7·77 (6·18)
Retic count ($\times 10^3$/μl)	139·4 (49·8)	130·9 (42·4)	−8·52 (38·9)	−1·42 (38·3)
RBC count ($\times 10^{12}$/l)	4·27 (0·81)	4·5 (0·83)	0·23 (0·43)	6·0 (9·77)
Total bilirubin (μmol/l)	74·37 (50·56)	64·89 (48·0)	−9·48 (13·15)	−14·59 (13·74)
LDH (U/l)	362·7 (107·4)	370·0 (132·78)	7·31 (58·39)	1·22 (19·14)

N = 8; first eight patients with NTD β-thalassaemia completing eight weeks of treatment. Hb, haemoglobin; LDH, lactate dehydrogenase; MCH, mean corpuscular haemoglobin; NTD, non-transfusion-dependent; RBC, red blood cell count; Retic, reticulocyte; Retic-Hb, cell haemoglobin content in reticulocytes; SD, standard deviation.

detailed above and highlights notable differences between the mouse model and the human disease. It is possible that in human β-thalassaemias, bitopertin treatment may have resulted in a greater reduction in all globin chain synthesis, due to reduced glycine availability,[7] and decreased haem availability, via the haem-regulated inhibitor regulatory mechanisms originally described by Chen in 2007.[8] Our study does not seem to indicate that the lack of therapeutic efficacy of bitopertin was due to inappropriately high dosages: in the patient who experienced the largest and most persistent increase in Hb (#2), dosage was increased to 120 mg/day (Figure S1) with no detrimental effects on Hb (Fig 1). Iron restriction leading to haematological improvement in the β-thalassaemia mouse model is usually accompanied by decreased serum iron, transferrin saturation and serum erythropoietin, in conjunction with reduced transferrin receptor expression on erythroblasts.[6,9] In this human study, no significant changes in serum erythropoietin or serum transferrin receptor were noted at Weeks 4 and 6 (data not shown), highlighting significant

pathophysiological differences compared with the mouse model.

Studies in the β-thalassaemia mouse model have shown that the iron chelator deferiprone abolishes the beneficial effects of bitopertin on anaemia and haemolysis.[6] We hypothesised that changes in intracellular iron (affected by iron chelation) might be important for drug activity. Overall, 5/12 patients were on iron chelation during the trial (Table SI). The only two patients treated with deferiprone during the study (#8 and #10) were among those who experienced the largest decrease in Hb values (Fig 1). The remaining three patients received other types of iron chelators, providing insufficient evidence from the human study to determine whether iron chelation may blunt response to bitopertin.

In conclusion, this study demonstrates that glycine restriction induced by bitopertin fails to improve erythropoiesis and RBC survival in NTD β-thalassaemia.

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Conflict of interest

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Ali T. Taher[1]
Vip Viprakasit[2]
Maria Domenica Cappellini[3]
Dominik Kraus[4]
Patrick Cech[4]
Dietmar Volz[4]
Erica Winter[5]
Stephane Nave[4]
Juergen Dukart[4,6,7]
Omar Khwaja[4]
Annette Koerner[4]
Ricardo Hermosilla[4]
Carlo Brugnara[8,9]

[1]Department of Internal Medicine, Division of Hematology and Oncology, American University of Beirut Medical Center, Beirut, Lebanon, [2]Department of Pediatrics and Siriraj Thalassemia Center, Division of Hematology and Oncology, Mahidol University, Bangkok, Thailand, [3]Department of Medicine, Ca'Granda Foundation IRCCS, University of Milan, Milan, Italy, [4]Roche Pharma Research and Early Development, Roche Innovation Center Basel, Basel, Switzerland, [5]Roche Pharma Research and Early Development, Roche Innovation Center New York, New York, NY, USA, [6]Institute of Neuroscience and Medicine, Brain & Behaviour (INM-7), Research Centre Jülich, Jülich, [7]Institute of Systems Neuroscience, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany, [8]Department of Laboratory Medicine, Boston Children's Hospital and [9]Department of Pathology, Harvard Medical School, Boston, MA, USA.
E-mail: ataher@aub.edu.lb

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Individual bitopertin dosing and treatment duration for patients with β-thalassaemia. Patients are sorted vertically according to study enrolment order. The dashed red vertical line depicts the time point of the preliminary efficacy analysis. *Denotes the time point for each patient at which the decision was taken to modify the study conduct by decreasing the target dose and eventually discontinue (patients #8, #9 and #10) or interrupt treatment for two weeks (patients #4, #6, #11 and #12). Dose levels depicted refer to daily dose.

Table SI. Clinical and haematological characteristics of enrolled patients.

Table SII. Treatment-emergent adverse events (AEs) observed in patients with non-transfusion-dependent (NTD) β-thalassaemia undergoing treatment with bitopertin.

Supporting Information

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Whole-blood CCR7 expression and chemoattraction in red blood cell alloimmunization

Understanding the mechanisms of the human immune response against red blood cell (RBC) antigens is a major issue for transfused patients.[1,2] $CD4^+$ T lymphocytes (TLs) play a key role in these reactions.[3,4] Previously described by our group and others, several information are available about TL phenotypes and functions [regulatory T cells (Treg), T-helper cells type 17 (Th17) and follicular helper T cells (Tfh)],[5–10] but little is known about the encounter of T cells with B cells leading to B-cell differentiation and antibody production in this context. T-cell migration has not been studied in RBC alloimmunization.

T-cell migration is essential for adaptive immune responses. T-cell motility is controlled by a combination of factors provided by the microenvironment and chemokines.[11] The autonomous migration of naïve T cells, analogous to a random walk, is supported by CC-chemokine ligand 19 (CCL19), CCL21 and CXC-chemokine ligand 12 (CXCL12).[11,12] The presence of CC-chemokine receptor 7 (CCR7), the receptor for CCL19 and CCL21, on T cells is required for the mobility of these cells. CXCL12 acts on T and B cells through its specific receptor, CXCR4.[11] CCR7 and CXCR4 do not work alone; CXCR5 also plays a major role in immune responses, promoting the $CXCR5^+$ TLs' circulation.[13] The T-box transcription factor (Tbet) induces CXCR3 expression,[14] and promotes the circulation of TLs.[13] Interestingly, we previously reported a significant 18-fold reduction in Tbet expression levels in the $CD4^+$ TLs of alloimmunized patients compared to non-alloimmunized patients.[5] Thus, with less Tbet expression, this may reduce the expression of CXCR3 and reduce the circulation of TLs. Moreover, we also described some difference in chemokine receptor expression between alloimmunized and non-alloimmunized sickle cell disease (SCD) patients: higher levels of CCR7 expression on T regulatory cells (Treg) in non-alloimmunized patients.[5]

Given the importance of T-cell migration for bringing T cells and B cells into contact, and based on our previous results,[5,9,10] we hypothesized that the phenotype and migratory function of $CD4^+$ T cells in SCD patients may differ according to anti-RBC alloimmunization response status. We therefore studied the chemokine receptor expression phenotype at the surface of $CD4^+$ TLs and Tregs, and the migration of these cells in response to CXCR4 ligand (CXCL12) and CCR7 ligands (CCL19 and CCL21), with patients recruited from the same cohort as we had already documented.[5,9,10] Two groups of polytransfused adult SCD patients were included in this study: alloimmunized and non-alloimmunized patients (Table SI and Figure S1).

Chemokine receptor expression was analysed on Tregs and conventional $CD4^+$ TLs in whole blood (Fig 1A). Treg cells were analysed as previously described.[9] No differences in expression between SCD patients and the control group were observed for CXCR3, CCR6 or CXCR5 (data not shown). Similarly, the expression of these three chemokine receptors was similar between SCD groups (data not shown). Individual analyses of these receptors did not highlight a possibility of using Th17 or Tfh cells to distinguish between alloimmunization statuses, as previously suggested.[8–10] Contrary to our