Proof of Mechanism Studies with Bitopertin, a Selective Glycine Transporter 1 Inhibitor Under Development for the Treatment of Erythropoietic Protoporphyria (EPP) and X-linked Protoporphyria (XLPP)

Vu Hong[1,4], Sarah Ducamp[2,4], Dean Campagna[3], Min Wu[1], Pavan Reddy[1], Brian MacDonald[1], Mark Fleming[2], Maria G. Beconi[1], Paul Schmidt[2]

[1]Disc Medicine Inc, 150 Cambridgepark Dr, Suite 103, Cambridge, MA 02140. [2]Department of Pathology, Boston Children's Hospital and Harvard Medical School, Boston, MA 02115
[3]Department of Pathology, Boston Children's Hospital, Boston, MA 02115. [4]Both authors contributed equally

INTRODUCTION

Bitopertin is a selective and orally available inhibitor of glycine transporter 1 (GlyT1), which supplies extracellular glycine for the initial step of heme biosynthesis in erythroid cells. Bitopertin has been studied extensively in clinical trials (>4,000 human subjects), has a favorable safety profile, and has been shown to modulate the heme biosynthesis pathway. Disc Medicine is developing bitopertin as a first-in-class, potentially disease modifying therapy for EPP and XLPP, two rare genetic disorders of heme biosynthesis. EPP is caused by a partial deficiency of the enzyme ferrochelatase (FECH), the last enzyme in the heme biosynthesis pathway that incorporates iron into protoporphyrin IX (PPIX). In EPP patients, a loss of >65% of FECH activity results in accumulation of PPIX, which leads to painful cutaneous photosensitivity with erythema and edema and hepatobiliary injury. In XLPP, mutations in the C-terminal domain of 5'-Aminolevulinate Synthase 2 (ALAS2), the first enzyme in heme production, result in a gain of function leading to elevated 5-aminilevulinic acid (5-ALA) production, and PPIX accumulation (Fig. 1A). We hypothesize that, by inhibiting glycine uptake into erythroid precursors, bitopertin can restrict heme pathway metabolite production and reduce the disease-causative PPIX accumulation in the blood of EPP and XLPP patients (Fig. 1B).

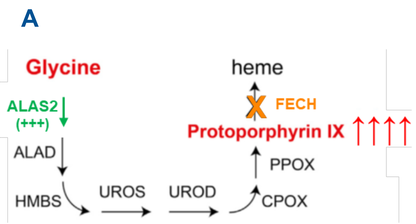


***Figure 1A**. Enzyme defects in EPP and XLPP lead to the accumulation of PPIX, a phototoxic metabolite*

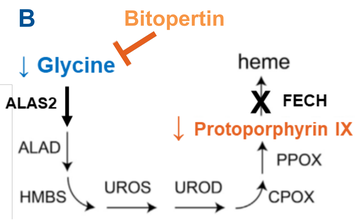


***Figure 1B.** Bitopertin, a GlyT1 inhibitor, reduces PPIX accumulation in EPP and XLPP*

OBJECTIVES

- To establish cellular models of EPP using human erythroleukemia K562 and human cord blood CD34+ cells
- To evaluate the effect of bitopertin on reducing PPIX accumulation in EPP cellular models
- To evaluate the effect of bitopertin on PPIX accumulation and hemoglobin levels in mouse models of EPP and XLPP

METHODS

- For K562 cells, we performed CRISPR-Cas9 genome editing to knock down one FECH allele and introduce the FECH c.315-48C hypomorphic variant of the EPP genotype in trans.
- For CD34+ cells, knock down (>60% reduction) of FECH mRNA was achieved with lentiviral vectors expressing shRNA of FECH. Transduced cells were differentiated for 9 days with Bitopertin or ORG-25543.
- The efficacy of bitopertin to treat EPP was further evaluated in female *Fech*^{m1Pas} EPP mouse model and in male *Alas2*$^{Q548X/Y}$ XLPP mouse model. The recessive *Fech*^{m1Pas} allele is an ethylnitrosourea (ENU)-induced missense mutation that retains approximately 5% residual ferrochelatase activity (Tutois et al, J Clin Invest. 1991; 88: p1730). The *Alas2*^{Q548X} animals were generated by employing CRISPR-Cas9 editing technology to introduce a known human gain-of-function mutation (S. Ducamp et al, poster). *Fech*^{m1Pas} and *Alas2*^{Q548X} mice were fed with diet containing 0 or 100 ppm bitopertin for 8 weeks starting at 6 weeks of age. Effects on PPIX and hemoglobin were determined at the end of 8 weeks of dosing.

RESULTS

Establishment of K562 cellular model of EPP

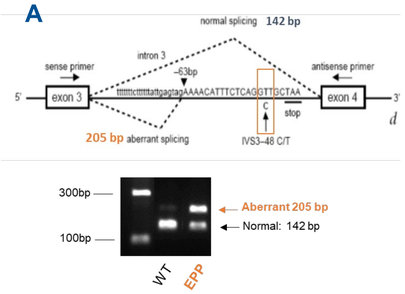


***Figure 2A.** The intronic IVS3–48T→C mutation increases the use of an aberrant splice site*

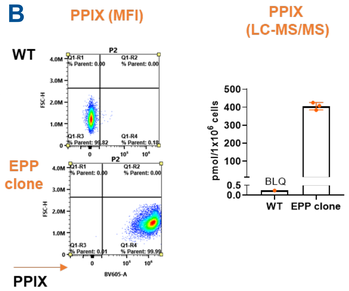


***Figure 2B.** IVS3-48C/KO mutations resulted in supra-physiological levels of PPIX*

Compound	K562 EPP assay (EC_{50}, nM)	GlyT1 uptake assay (IC_{50}, nM)*
Bitopertin	**9**	25
PF-03463275	**46**	12
ALX-5407	**0.34**	3
ORG-24598	**5.6**	7
LY-2365109	**4.1**	16
ORG-25543 (GlyT2 inhibitor)	**no inhibition**	>10,000

** Literature values*

***Table 1.** Activity of known GlyT1 and GlyT2 specific inhibitors in the K562 cellular model of EPP*

Bitopertin reduced PPIX in K562-EPP cell model

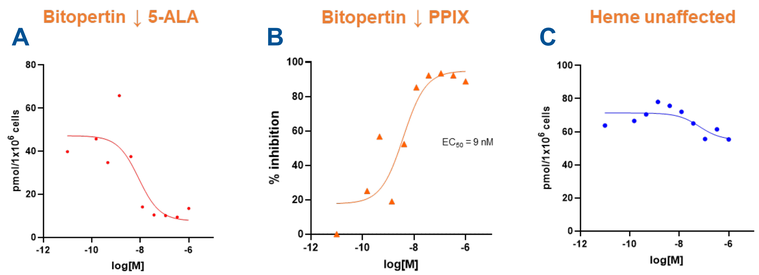


***Figure 3.** Bitopertin decreased formation of 5-ALA (A), prevented PPIX accumulation in a dose dependent manner (B), without affecting heme formation (C)*

Bitopertin reduced PPIX in hCD34+ model of EPP

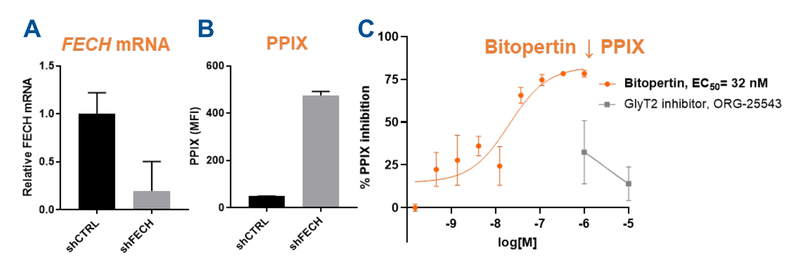
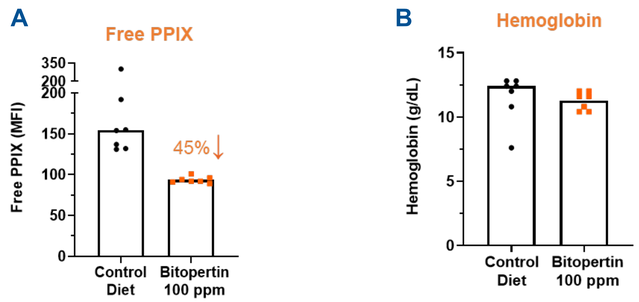


***Figure 4.** ~75% reduction in FECH mRNA level observed (4A); leading to PPIX accumulation in cells (B)*

***Figure 4C.** Bitopertin reduced PPIX with an EC_{50} of 32nM, while Gly2 inhibitor ORG-25543 had minimal effect*

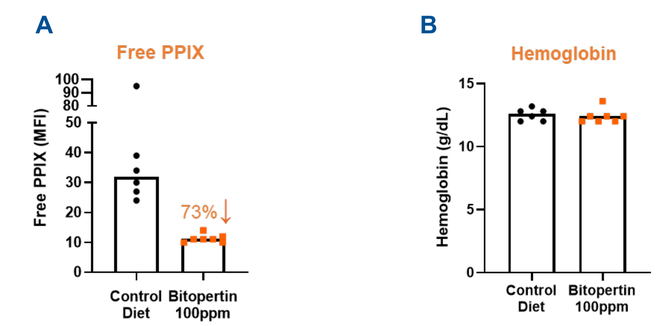
Bitopertin reduced PPIX in *Fech*^{m1pas} mice



Bar height represents median, symbols represent individual values

***Figure 5.** Effects of bitopertin in EPP mouse model (Fech^{m1pas} /Fech^{m1pas}) on PPIX (A) and hemoglobin levels (B) after 8 weeks of treatment on 100ppm bitopertin diet*

Bitopertin reduced PPIX in *Alas2*^{Q548X} mice



Bar height represents median, symbols represent individual values

***Figure 6.** . Effects of bitopertin in XLPP mouse model (Alas2$^{Q548X/Y}$) on PPIX (A) and hemoglobin levels (B) after 8 weeks of treatment on 100ppm bitopertin diet*

CONCLUSIONS

- Bitopertin is a selective GlyT1 inhibitor with a well-characterized safety profile in humans
- Bitopertin reduced PPIX in K562 and hCD34+ cellular models of EPP
- Bitopertin reduced PPIX in mouse models of EPP and XLPP without effects on hemoglobin. Target reduction of 30-50% exceeded.
- Bitopertin has the potential to improve light tolerance and hepatobiliary injury in EPP patients by decreasing PPIX in erythrocytes

CONTACT INFORMATION

Vu Hong, vhong@discmedicine.com

disc medicine

I APPROVE THE SUBMISSION OF THIS ABSTRACT WITH THE AUTHORS LISTED FOR SUBMISSION TO HEME BIOSYNTHESIS AND THE PORPHYRIAS 2021 SYMPOSIUM

NAME	Signature	Date
Vu Hong	DocuSigned by: Vu Hong 12EB93D4F793401...	
Sarah Ducamp	DocuSigned by: Sarah Ducamp 4329B1519D12448...	
Dean Campagna	DocuSigned by: Dean Campagna F993A3BC6EDF41E...	
Min Wu	DocuSigned by: Min Wu 00AB434335CA49A...	
Pavan Reddy	DocuSigned by: Pavan Reddy AE760D72F2F5471...	
Brian MacDonald	DocuSigned by: Brian MacDonald FAF6091578E44C9...	
Mark Fleming	DocuSigned by: Mark Fleming 68E129AC8C77413...	
Maria G. Beconi	DocuSigned by: Maria G. Beconi 704373BC94D540F...	
Paul Schmidt	DocuSigned by: Paul Schmidt 130BF449447A44C...	
Jonathan Yu	DocuSigned by: 2D2CED152733497...	
John Quisel	DocuSigned by: John Quisel D74EF6B7011742E...	