Pre-Analytical Considerations Are Important In The Pharmacological Assessment Of Enzyme Therapeutics

Kevin Thorneloe, Chris Daige, Jason Wiggins, Leslie Priddy, Jess Van Cleef, Silvia Ferrati, Leslie Sloan, and Scott Rowlinson

Introduction

Enzyme therapeutics with high catalytic activity and/or at high doses can have a unique ability to continue metabolizing their target analyte even after blood collection. This poorly characterized phenomenon may lead to an artifactual overestimation of the *in vivo* pharmacological effects of an enzyme therapeutic, and subsequently result in an adverse determination of:

a) The relationship between the pharmacodynamic (PD) change in a metabolite to any efficacious or toxicological effects in non-clinical studies

b) Starting doses for first in man clinical trials

c) The relationship between a PD change in a metabolite and therapeutic benefit in the clinic

With the recent development of highly effective metabolite lowering enzyme therapeutics, we have examined the impact of pre-analytical processing as a source of artifactual metabolite measurements impacting pharmacological evaluation of non-clinical and clinical studies for the following programs:

a) Pegzilarginase, which is a pegylated modified human arginase 1 enzyme in clinical development for Arginase 1 Deficiency

b) AGLE-177, which is a novel, engineered human enzyme with specificity for homocysteine and homocystine which is in development to treat patients with Homocystinuria

Results

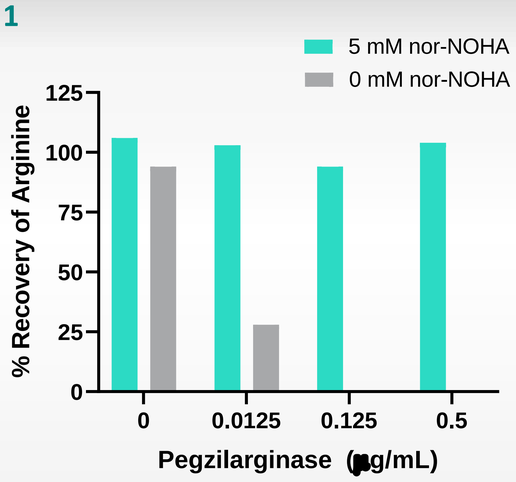
Pegzilarginase

Investigation of the potential for artifactual lowering of arginine levels due to continued enzyme activity *ex vivo*

Ex vivo studies demonstrated (Fig 1):

- Substantial reduction in the % of arginine recovery following spiking of pegzilarginase into whole blood
- Inhibition of this effect using nor-NOHA[1] as an *ex vivo* inhibitor of pegzilarginase activity

Figure 1

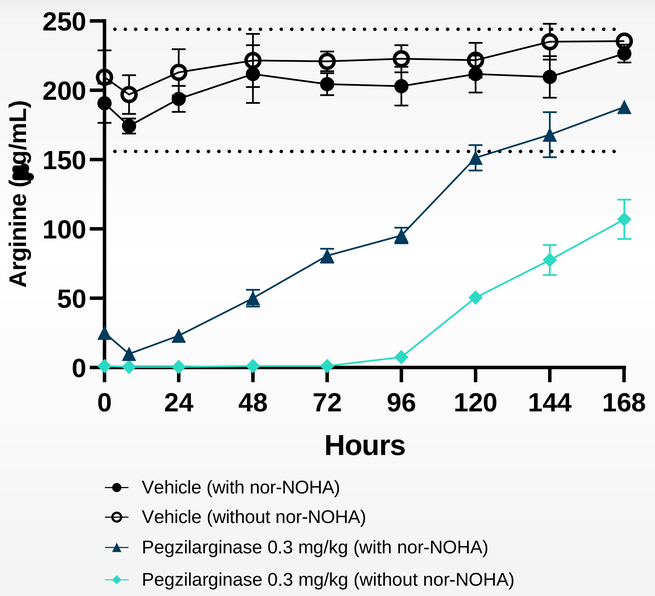


Whole blood from healthy volunteers was collected and aliquoted (1ml) into tubes with and without 5 mM nor-NOHA inhibitor and 25 ug of arginine (Arg). Aliquots were subsequently spiked with pegzilarginase to 0.0125, 0.125, and 0.5 mg/mL. After incubation on ice for 30 min, samples were processed to plasma and treated with 25 ul of glacial acetic acid prior to Arg quantification by LC-MS/MS.

Effective pre-analytical control of residual pegzilarginase enzyme activity allows accurate determination of *in vivo* pharmacological effects in non-clinical studies

The PD profile of pegzilarginase effects on plasma arginine levels was investigated in a non-clinical study in normal rats in the presence and absence the nor-NOHA inhibitor (Fig 2). In the absence of the inhibitor, the magnitude of the reduction in plasma arginine was substantially greater than with inhibitor, leading to an over-estimation of the PD effect with no inhibitor present.

Figure 2



Dotted lines represent the upper and lower normal range from non-treated animals

Juvenile male rats were dosed weekly with pegzilarginase at dose levels of 0 and 0.3 mg/kg for 6 weeks. Whole blood was collected into tubes +/- nor-NOHA at various time intervals over the course of a week after the 6th dose. After plasma was processed to plasma and acidified, arginine content was assessed by LC-MS/MS.

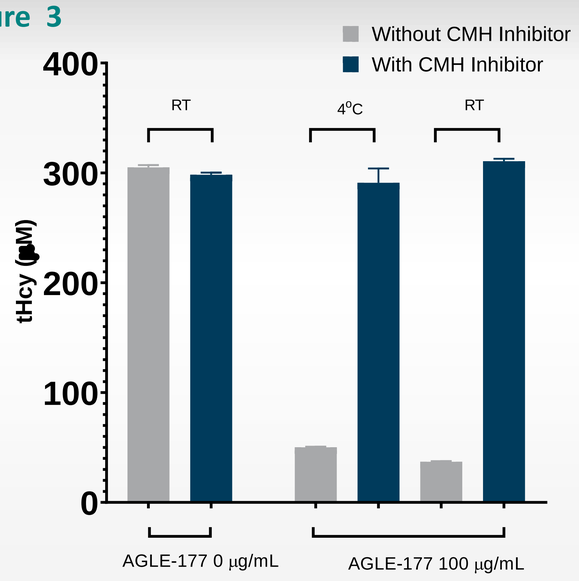
AGLE-177

Investigation of potential artifactual total homocysteine (tHcy) level lowering due to continued enzyme activity *ex vivo*

Ex vivo studies demonstrated (Fig 3):

- Substantial reductions in the amount of tHcy recovered following spiking of AGLE-177 into whole blood with >250 µM of artifactual metabolism when the CMH:HCl inhibitor[2] was not used during sample processing
- Inhibition of this effect using CMH:HCl[2] as an *ex vivo* inhibitor of AGLE-177 activity

Figure 3



Whole blood collected from wild-type mice was spiked with 150 µM homocystine followed by 100 µg/mL AGLE-177. Aliquots were immediately transferred to tubes with and without CMH:HCl to a final concentration of 50 mM. Samples were incubated at room temperature (RT) or on ice (4°C) for 30 min prior to processing to plasma and acidification. tHcy content was determined via LC-MS/MS.

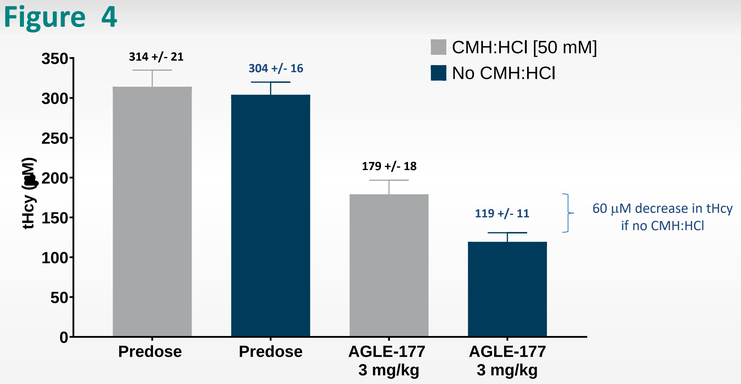
Effective pre-analytical control of residual AGLE-177 enzyme activity allows accurate determination of *in vivo* pharmacological effects in non-clinical studies

CBS knockout mice efficacy study

The PD profile of AGLE-177 effects on plasma tHcy levels was investigated in a non-clinical study in CBS knockout mice in the presence and absence the CMH:HCl inhibitor (Fig 4)

Analysis of tHcy concentrations showed an additional 60 µM of artifactual metabolism when CMH:HCl inhibitor was absent from sample processing. This again demonstrates that in studies of potent enzyme therapeutics, plasma analysis without an inhibitor leads to an over-estimation of important PD effects.

Figure 4

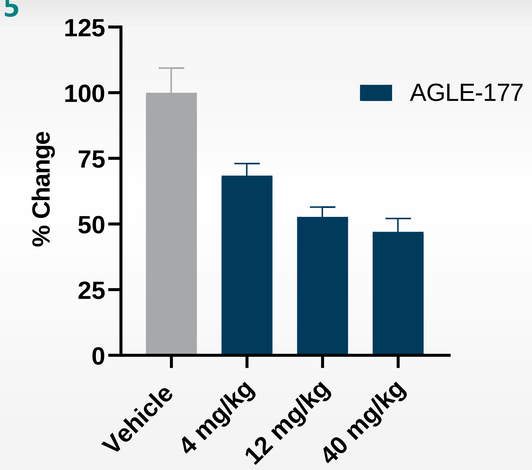


Baseline blood was collected from CBS KO animals (n=11). Following 1 week recovery, mice were dosed with 3 mg/kg AGLE-177 and blood collected into tubes with and without 50 mM CMH:HCl. After processing to plasma and acidification, values of tHcy in CBS KO mice were determined.

Normal rat toxicology studies

The PD profile of AGLE-177 effects on plasma tHcy levels was investigated in a non-clinical study in normal rats in the presence of the CMH:HCl inhibitor (Fig 5). These data reflect robust, statistically significant, *in vivo* reduction of tHcy in wild type animals and the use of an inhibitor pre-analytically ensures that these data are indicative of *in vivo* activity and not an artifact driven by *ex vivo* metabolism.

Figure 5



Sprague Dawley rats with normal levels of tHcy were dosed once weekly with 4, 12, or 40 mg/kg IV with AGLE-177 for 13 weeks. Samples were taken 24 hrs after dosing in tubes containing CMH:HCL, processed to plasma, acidified and analyzed for tHcy via LC-MS/MS.

Conclusions

1. Pegzilarginase and AGLE-177 show significant degradation of substrate during pre-analytical processing in the absence of inhibitor
2. Effective pre-analytical control of *ex vivo* enzyme activity was illustrated to be essential for accurate *in vivo* pharmacodynamic measurements
3. Accurate pharmacodynamic measures are critical for pre-clinical and clinical decision making related to safety, pharmacology, and data interpretation

References: 1= PMID: 10637120 , 2= PMID: 23488457

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Disclosures: *Employees of Aeglea BioTherapeutics Inc. have an equity interest in the company*