Abstract # 713.15

Creation of brain-penetrant iduronidase by conjugation with Angiopeps and demonstration of brain enzyme activity in a mouse model of MPSI

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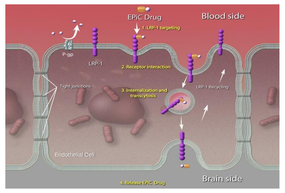
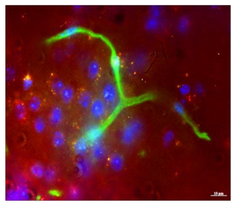
BACKGROUND

Angiochem is a clinical-stage biotechnology company discovering and developing new breakthrough drugs that are uniquely capable of crossing the blood brain barrier (BBB) to treat brain diseases. These new drugs have the potential to address significant medical needs, many of which cannot be effectively addressed due to the fundamental physiological challenge the BBB presents.

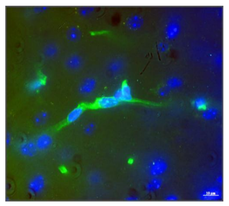


Using LRP1 receptor-mediated transcytosis has a number of inherent biochemical advantages for drug transport across the BBB, including high capacity, rapid turnover, recognition of numerous ligands, and limited down-regulation. We have created peptides (Angiopeps), including Angiopep-2 (An2), using a library based on binding sequences of known LRP-1 ligands.

Angiopeps cross the BBB



Angiopep-2

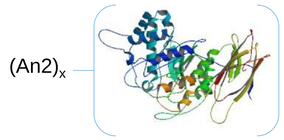


Control Peptide

- Peptides labeled with Cy5.5
- Capillaries stained with vessel green (FITC-Lectin)
- Nuclei of brain cells stained with DAPI blue

Mucopolysaccharidosis I and IDUA enzyme replacement

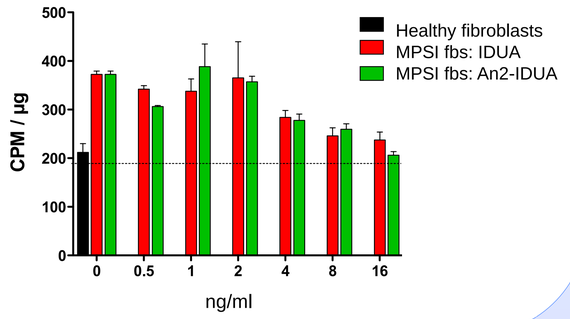
- MPSI is one of many rare lysosomal storage disorders arising from mutations in the genes encoding lysosomal enzymes. Absence of enzyme activity results in storage of substrate molecules such as carbohydrates and lipids, in the case of the MPS diseases, gangliosides (GM2, GM3) and glycosaminoglycans (GAGs) accumulate in tissues.
- Enzyme replacement therapy has been successful in treating peripheral symptoms and carbohydrate storage in MPS disorders; Aldurazyme is approved for treating MPS1.
- Because BBB penetration is limited for large molecule biologics such as enzymes, benefits of enzyme replacement therapy are limited to peripheral organs, while CNS symptoms remain untreated.
- Conjugation of Angiopeps to iduronidase (IDUA) results in a brain-penetrant enzyme that is suited to enzyme replacement therapy to treat CNS as well as peripheral symptoms.



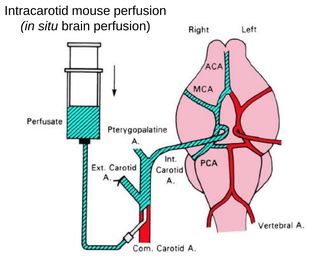
Conjugate Optimization

- Over 20 chemical conjugates were generated between Angiopeps and IDUA, with variation of the following parameters:
  - Angiopep sequence
  - Linker structure
  - Angiopep:Linker:IDUA ratio
  - Reaction conditions
- Conjugates were also generated by N-term or C-term fusion
- Conjugates were tested for the following properties
  - IDUA enzyme activity
  - IDUA uptake in to MPSI patient fibroblasts
  - Rate of entry into brain (intra-carotid artery delivery)
  - Brain enzyme activity in IDUA KO mice (ic or iv delivery)
  - Reduction of GAG levels in brain and peripheral tissues
  - Reduction of GM3 in brain (IHC)

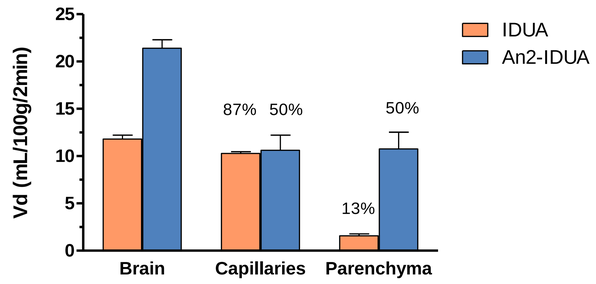
Cellular Activity: An2-IDUA normalizes GAG storage in MPSI patient fibroblasts



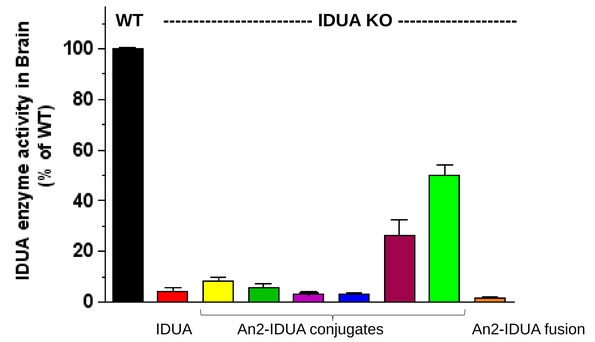
An2-IDUA reaches brain parenchyma



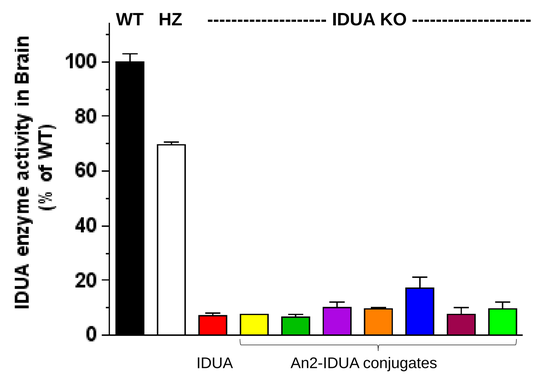
Measurement of [^{125}I] IDUA and An2-IDUA following *in situ* brain perfusion and capillary depletion demonstrates that native IDUA is predominantly associated with brain vasculature, whereas An2-IDUA is able to reach brain parenchyma.



IDUA activity in brain of MPSI KO mice



IDUA KO mice were treated with IDUA or An2-IDUA conjugates, 240 nM, infused via carotid artery over a 4 minute period. Brains were removed and homogenized for enzyme activity assay using fluorogenic 4-methyl-umbelliferyl a-L-iduronide as the substrate. Note that the enzymatic activitiy conferred by the fusion An2-IDUA protein is lower than that of native IDUA. The best conjugate restores 50% of WT activity.



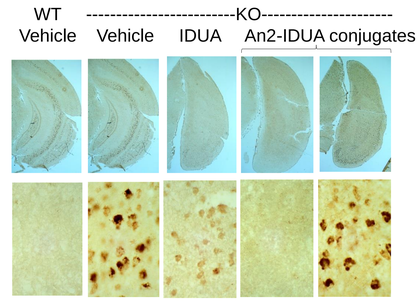
IDUA KO mice were treated with IDUA or An2-IDUA conjugates, 3 mg/kg, iv. After 2 hours, brains were removed and homogenized for enzyme activity assay using fluorogenic 4-methyl-umbelliferyl a-L-iduronide as the substrate. The best conjugate restores 17% of WT activity.

Note: Human studies suggest that correction of substrate storage is achieved with 1-3% of WT enzyme activity in the periphery.

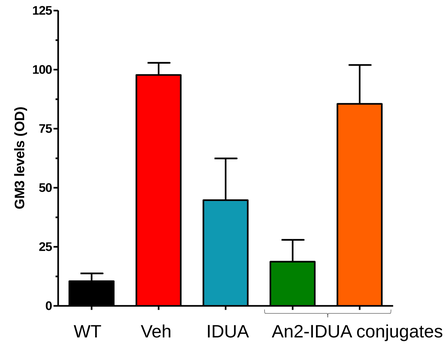
Reduction in brain GM3 storage by an An2-IDUA conjugate

Assessment of GM3 staining in brains of IDUA KO mice. IDUA KO mice were treated with vehicle or 3 mg/kg, iv enzyme (IDUA or An2-IDUA derivatives) once weekly for four weeks. Brains were removed and sectioned on a cryostat for immunostaining for GM3. High levels of GM3 were evident in the entorhinal cortex.

IHC



OD Quantitation



Early data indicate that brain GM3 storage is reduced by one of the An2-IDUA conjugates to a greater degree than by native IDUA. The lack of effect of a second An2-IDUA conjugate underscores the importance of testing multiple conjugates to optimize activity.

Conclusions

- **Conjugation of Angiopeps, LRP-1 targeting peptides, to IDUA results in brain-penetrant lysosomal enzymes.**
- **An2-IDUA conjugates retain enzymatic activity and ability to enter MPSI fibroblasts to normalize GAG levels.**
- **An2-IDUA conjugates enter brain parenchyma more efficiently than native IDUA, demonstrated by in situ brain perfusion followed by capillary depletion.**
- **Systemic administration of An2-IDUA conjugates results in brain IDUA activity of up to 50% of WT activity.**
- **Thus far, fusion constructs of An2-IDUA have been inferior to chemical conjugates; additional fusion protein variations will be necessary to optimize this approach.**
- **After 4 weeks of administration, GM3 levels in brains of IDUA KO mice were reduced for one conjugate.**
- **These results demonstrate the utility of the Angiopep strategy to create brain penetrant biologics for CNS disorders.**

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