Abstract Number: LPB0006

Recombinant Heparin: An Old Drug for the Modern World

Bryan E. Thacker[1], Kristen Thorne[1], Colin Cartwright[1], Jeeyoung Park[1], Kimberly Glass[1], Annie Chea[1], Walter Jeske[2], Jeanine Walenga[2], Susan Sharfstein[3], Jeffrey Esko[4] and Charles A. Glass[1]

[1]TEGA Therapeutics, San Diego, CA, USA; [2]Loyola University Chicago Health Sciences Campus, Maywood, IL, USA; [3]SUNY Polytechnic Institute, Albany, NY, USA; [4]University of California, San Diego, CA, USA

bthacker@tegatherapeutics.com

Conclusions

- Heparin is an essential drug sourced from animal tissues placing the supply at risk
- All cells produce heparan sulfate but heparin is produced specifically by mast cells
- Engineered mastocytoma cells produce heparan sulfate with anticoagulant potency exceeding that of unfractionated heparin
- Producing recombinant heparin is a feasible alternative to animal sources

1. The Heparin Problem

Heparin has been used in the clinic to manage blood clotting since the 1930s and is included on the World Health Organization's list of essential medicines. Hundreds of thousands of doses are administered in the US each day.

- Heparin is purified from porcine intestinal mucosa
- Most of the world's supply comes from animal populations in China
- The supply depends on the health and abundance of the pig population
- Widespread disease in pig populations limited supply in 2008
- Accompanying adulteration of crude heparin resulted in >250 deaths (1)


The New York Times
U.S. Identifies Tainted Heparin in 11 Countries
By GARDINER HARRIS APRIL 22, 2008

- African swine fever in 2018 cut China's pig population by one-third

Continued dependence on an animal population puts the supply of heparin at risk.

2. Recombinant Biotherapeutic Production

Recombinant DNA technologies have enabled production of protein therapeutics under scalable, GMP conditions, independent of animal products. Despite these advances, heparin continues to be produced from animals.

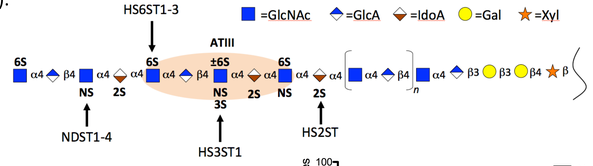
- Heparin is a highly sulfated polysaccharide
- Heparin is produced in a biosynthetic pathway involving dozens of enzymes
- Heparin is made specifically by mast cells which cannot be readily cultured
- Other mammalian cells make heparan sulfate with lower sulfate content and little anticoagulant potency by the same biosynthetic pathway

Our objective is to modify the heparan sulfate biosynthetic pathway in cultured cells to make a heparin-like product with high anticoagulant activity that can be scaled as an alternative to porcine-derived heparin.

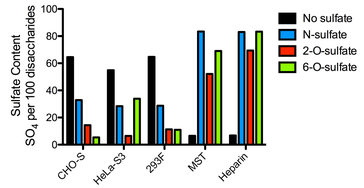
3. Mastocytoma Cells

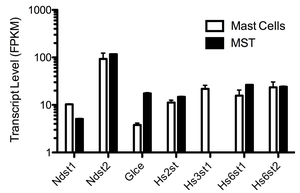
Producing recombinant heparin requires engineering the heparan sulfate biosynthetic pathway (2).

- Glycosyltransferases polymerize a chain of repeating *N*-acetylglucosamine (GlcNAc) and glucuronic acid (GlcA) disaccharide units
- A C5 epimerase converts some GlcA to iduronic acid (IdoA)
- Sulfotransferases (Ndst1-4, Hs2st, Hs3st1-6, Hs6st1-3) install sulfate groups at specific positions
- Biosynthesis is non-template driven
- Composition is determined largely by the expression level of biosynthetic enzymes
- A specific pentasaccharide sequence including *N*-, 3-*O*- and 6-*O*-sulfate is required for anticoagulation via binding and activation of antithrombin (ATIII) (3).



Mammalian cells commonly used for bioproduction make heparan sulfate with much lower sulfate content than heparin and had no anticoagulant potency. A naturally arising mastocytoma (MST) was isolated from mice. The cells grow readily in culture. (4) Heparan sulfate analysis showed that MST cells produce heparan sulfate with sulfate content similar to heparin but with very low anticoagulant activity.





RNAseq transcription analysis showed that MST cells lacked Hs3st1 compared to mast cells differentiated from mouse bone marrow derived cells. Disaccharide analysis also showed that 6-*O*-sulfate content was lower in MST heparan sulfate compared to heparin.

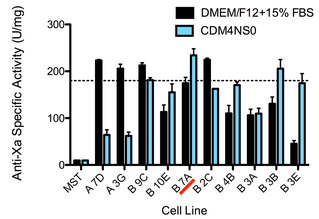
4. Engineering Recombinant Heparin

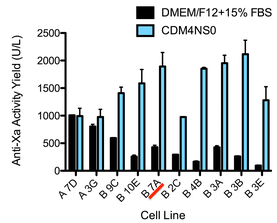
The heparan sulfate biosynthetic pathway of MST cells was genetically engineered using lentiviral transduction in DMEM/F12+15% FBS to increase anticoagulant potency.

- Hs3st1 was overexpressed alone and in combination with Hs6st1, Hs6st2 and/or Ndst2
- Single cell colonies were genotyped by PCR
- Heparan sulfate anti-Xa activity produced in DMEM/F12+15% FBS was assayed (see Table)

	Engineered MST Genotype	Colonies Tested	Average anti-Xa Activity per Well (mU/well)	Average anti-Xa Activity per Lysate Protein (mU/mg)
A	+Hs3st1	181	413	4.78
B	+Hs3st1+Hs6st1	190	523	4.27
C	+Hs3st1+Hs6st2	28	160	0.94
D	+Hs3st1+Ndst2+Hs6st1	16	155	2.32

Top colonies were selected from transfections A and B to determine anti-Xa specific activity and total activity yield of heparan sulfate in DMEM/F12+15% FBS and CDM4NS0 (serum free). Cell lines transduced with Hs3st1 only had lower activity in CDM4NS0. Cell line B7A was chosen because of its high activity in chemically defined medium.



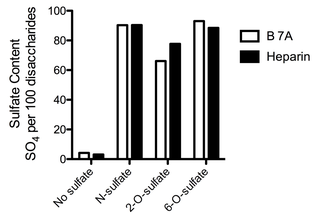


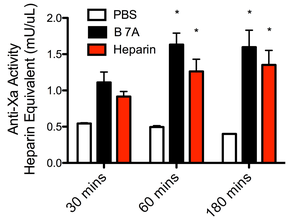
5. Characterizing Recombinant Heparin

Heparan sulfate was produced from MST B7A cells grown in CDM4NS0. The anti-Xa/anti-IIa specific activity, sulfate content and average chain length was determined and compared to porcine derived unfractionated heparin (UFH).

3 mg/kg UFH and B 7A heparan sulfate were injected subcutaneously into mice (n=4 per group). Blood was collected from the mice at 0.5, 1 and 3 hours post injection.

	B 7A	UFH
Anti-Xa activity (U/mg)	297	197
Anti-IIa activity (U/mg)	311	209
Anti-Xa/Anti-IIa	0.95	0.94
Chain size Mw (kDa)	26.4	17.1





References

(1) H. Liu, Z. Zhang & R. Linhardt. Nat Prod Rep, 2009. (2) J. Kreuger & L. Kjellen. J Histochem Cytochem, 2012. (3) D. Atha et al. Biochemistry, 1985. (4) R. Montgomery, et al. PNAS, 1992.

Acknowledgements

This work was funded by NSF SBIR grant to C.A.G.