Targeting Distinct Tau Strains and Tau Aggregate Sizes with Heparin and Heparinoids to Explore Differential Inhibition of Cell Uptake and Seeding

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Background

Tauopathies (including Alzheimer's Disease) are incurable, progressive neurodegenerative diseases caused by tau protein aggregation. Evidence suggests that tau aggregates spread pathology as do prions, infectious proteins that transmit a pathologic conformation to native proteins via strains (which are disease-specific conformers that propagate indefinitely in living systems). Like prion protein, tau also forms strains. It has already been observed in animal models that heparin mimetics inhibit prion pathogenesis and that genetic knockdowns within the heparin sulfate proteoglycan (HSPG) pathway reduce tau pathology. To better understand whether distinct tau strains are preferentially inhibited by certain heparinoids (which would have important implications for the design of targeted therapies) and if different strains interact differently with cell surface HSPGs (which could help explain varying pathological behavior between strains), I explored whether heparin sensitivity differs by 1) tau strain, 2) tau aggregate size, and 3) heparinoid size and structure.

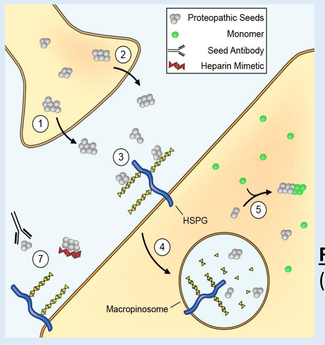


Figure 1: Tau aggregates spread pathology by binding cell surface HSPGs. Uptake into cells can be inhibited by heparin and its derivatives which are well known mimics of HSPG (Holmes et al., JBC 2014).

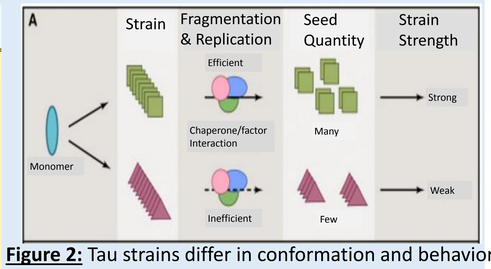


Figure 2: Tau strains differ in conformation and behavior (Sanders, Kaufman, et al., Neuron, 2016).

Figure 3: The Diamond lab has identified 19 different strains of tau (Sanders, Kaufman, et al., Neuron, 2014).

Methods

Tau uptake and seeding was measured using a HEK P301S "biosensor" cell line which allowed for intracellular aggregates to be measured using FRET flow cytometry. Experimental methods are as follows:

1. SEC was used to clean cell lysate containing tau aggregates.
2. Tau aggregates were incubated with heparin (0.2, 2, 20, & 200 ug/mL) for 24-hours prior to addition to cultured cells.
3. After a 48-hour incubation period, cells were washed, dissociated from the tissue culture dish, and fixed in paraformaldehyde.
4. Flow cytometry was used to measure tau seeding.

Methods

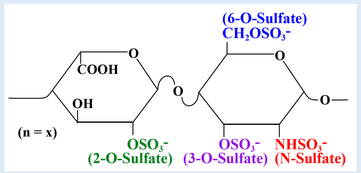
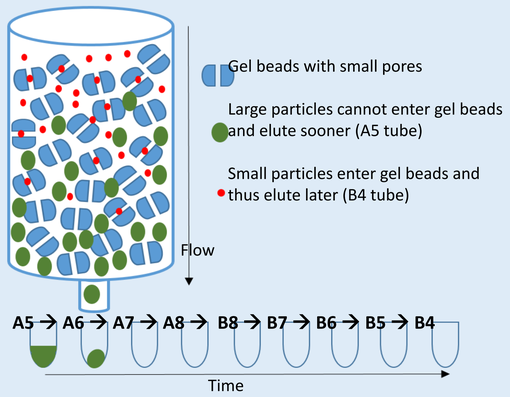


Figure 4: Chemical structure of a heparin disaccharide unit. Heparin is composed of approximately 40 such units. Note the various sulfate moieties. (Image modified from Brandon Holmes)

Figure 5: Size Exclusion Chromatography (SEC)



Results

1. Tau seeding was comparably inhibited by heparin regardless of tau aggregate size.

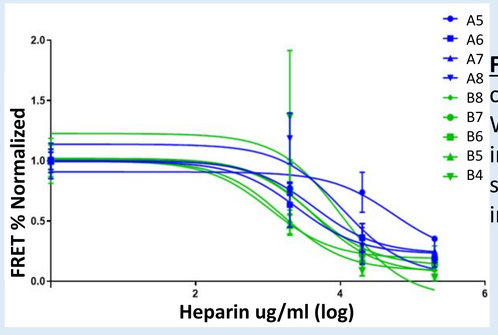


Figure 6: Tau aggregates from Clone 9 cell lysate were separated using SEC. When tested for heparin inhibition of intracellular seeding, results showed no significant differences in seeding inhibition based on tau aggregate size.

2. Different tau strains appear to have varying sensitivity to heparin.

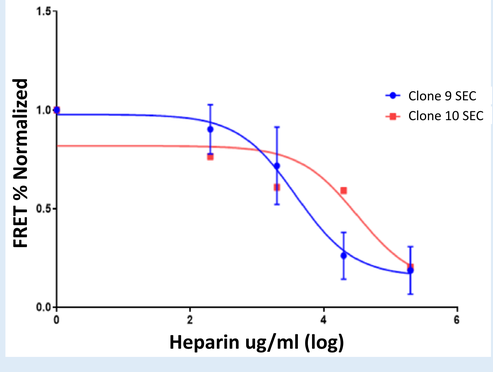


Figure 7: Two different strains of tau (Clone 9 and Clone 10) were tested for heparin inhibition of intracellular seeding. The results showed varying sensitivity to heparin (Clone 9 IC_{50} = 335.9nM vs Clone 10: IC_{50} = 2.1uM).

Results

3. Diverse heparinoids have highly variable inhibition of tau seeding, some having no effect and some having an effect nearly as strong as heparin.

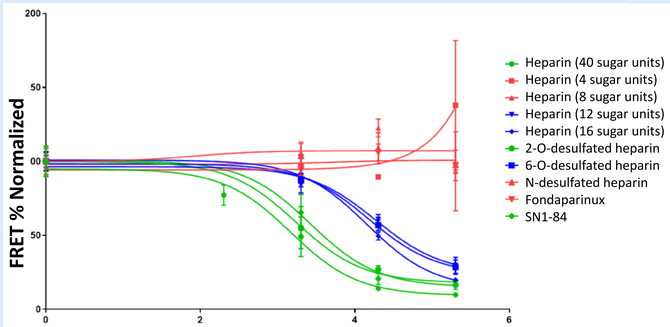


Figure 8:
- Heparin (40 units) had the strongest inhibitory effect on tau seeding.
- Larger heparinoids better inhibited seeding (40>16>12>8>4).
- The N-sulfate group is the most important moiety for tau binding.
- The 6-O-sulfate group is the 2nd most important moiety for tau binding.
- The 2-O-sulfate group is the least important moiety for tau binding.

Conclusions

This data suggests that:

1. Tau seeding is similarly inhibited by heparin regardless of tau aggregate size.
2. Seeding of different strains of tau may be variably inhibited by heparin, hinting that specificity and avidity may differ by strain.
3. Certain size and sulfation patterns of heparin affect seeding inhibition differentially, supporting the idea that crucial binding domains on heparin are necessary for pathologic tau spread between cells. This matches other data produced in the lab using genetic knockouts.

If true, this knowledge will be applicable across many tauopathies and may influence diagnosis (because identification of tau strains can differentiate pathology) and treatment (strain-specific therapies may be required).

Acknowledgements

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