ABSTRACT

One of the key limiting parameters in the penetration and effectiveness of topically applied ocular therapy is the mean residence time for protein drugs. The problem becomes more acute due to the additional burden of reduced penetration through the cornea and conjunctiva. Residency time might be increased however by fusing a protein to a mucosal surface binding protein. Gal-3 is a small pentameric mucosal surface resident protein that cross-links O-type mucins. We have demonstrated that fusion of a protein to the small sugar-binding domain of gal-3 increases the half-life of its ocular surface residence by at least 6-fold. This might afford a means to increase the absorption of biologics from the ocular surface, reducing either the dose required, the frequency of administration, or both.

BACKGROUND

Short residence time limits the penetrance and effectiveness of topical ocular therapies. Most solutions clear from the eye through drainage, with first-order clearance about 4 times that of bulk tear flow. For example, sodium hyaluronate has a residence half-life of 321 seconds, and polyvinyl alcohol just 39 seconds. Approaches for increasing pre-corneal residence time include increasing formulation viscosity, receptor targeting, and use of mucoadhesives. (Cleeman 1992 Jul;11(6):288-93.)

Clearance becomes more troublesome for protein drugs due to their reduced penetration. We reasoned that residence time for a protein drug could be increased by fusing it to a mucosal surface binding protein such as galactin-3, a small, pentameric mucosal surface resident protein that crosslinks O-type mucins.

The Corneal Glycocalyx and Galectin-3

- The corneal epithelium is protected by a glycocalyx (mucus) layer consisting of transmembrane mucins with O- and N-linked glycosylation
- Galectin-3 multimers crosslink mucins to create a diffusion barrier (Argüeso et al. JBC 2009)
- In vivo, glycocalyx disruption leads to increased corneal penetration.

Can we use galactin-3 as a mucoadhesive moiety to increase corneal residence time of topically administered biologics?

Gal-3 Fusion Proteins

- Gal-3 Fusion Proteins are soluble β-galactoside-binding lectins that regulate cell signaling. Galactin-3 has a unique (amongst galectins) N-terminal domain that mediates self-association
- We used the galactin-3 C-terminal domain (“Gal”) as a monomeric, glycosynthetically inert moiety that is stable to proteolysis
- We chose Gaussia luciferase (Luc) as a model protein for transduction into cells
- Initially compared effects of single or double gal domains

Gal Safety

Galactin-3 has been implicated as a cardiovascular risk factor (de Boer et al. J Curr Heart Fail Rep 2010). An oncogene/metalloenzyme risk factor (Zhao et al. Cancer Res 2010). These effects derive from the ability Galactin-3 to oligomerize and cluster receptors at the cell surface, driving ligand-independent signaling (see fig).

CONCLUSIONS

- Fusion of a protein to the small sugar-binding domain of galactin-3 increases the half-life of its ocular surface residence to 30 minutes by binding to cell surface glycans
- Gal potentiates the action of linked cell-surface acting cytokines by almost 2 orders of magnitude, possibly by increasing the affinity to the cell surface receptor. This suggests that the potentiation may be cytokine-specific, which we are exploring.

We may therefore have found a general way to increase the performance of biologics at the ocular surface, reducing either the dose required, the frequency of administration, or both.

In vitro Data

EULSA Binding to Asialofetuin:
- Plate coated with a solution of 4mg/ml asialofetuin
- Blocked for 1 hour
- Protein incubated in the wells for 1 hour before wash and measurement
- Compared to Luc alone, one and two gal domains increase retention by 31- and 265-fold, respectively

In vivo Data

Gal Potentiates Cytokine Activity

To test whether fusion to gal adversely affects activity of the fused moiety, we fused gal to our 5-1 antigen, ST2, using QAS13 linker, in the following constructs:

- Purified proteins were tested in a HEK Blue LTR reporter cell-based assay (transduction)
- Cells were seeded at 55,000 cells/ml onto a 24 well plate and mixed with 0.1ng/ml 5-1 and dilutions of the 530Q protein.
- After 24h, activation of 5-1/F with measurement using Quanti-Blue substrate for secreted alkaline phosphatase.
- Antigenic potency of 530Q had increased 75-fold.


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