Modulation of Galectin-3 – TREM2 Interactions as a Potential Treatment for Neurodegenerative Diseases

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Introduction

Neurodegenerative diseases (ND) are debilitating, progressive conditions with few disease-modifying treatments. Neuroinflammation is a major factor in the pathogenesis of many ND; however, broad-acting anti-inflammatory drugs have generally been ineffective in clinical trials.[1,2] Microglia, the innate immune cells of the brain, can assume inflammatory, homeostatic, and disease-associated phenotypes.[3-5] Targeting microglia as a potential therapy is complex, as these cells play both beneficial and detrimental roles in the progression of ND. It is likely that non-specific targeting of the neuroprotective function of microglia is responsible for the failure of most anti-inflammatory drugs.[1] Galectin-3 (Gal-3) is a chimeric β-galactoside-binding lectin directly involved in multiple pathologies and is reported to have a significant role in inflammation and fibrosis in several ND.[6-8] Gal-3 is an endogenous ligand for the microglial receptor triggering receptor expressed on myeloid cells 2 (TREM2), which is genetically associated with multiple ND and is critical for the modulation of microglial activation towards neuroprotection.[9-10] Gal-3 is a strong pharmacological target for multiple ND; however, Gal-3 inhibitors currently in clinical trials for non-neurological disorders are large, carbohydrate-based molecules that show low specificity for Gal-3 and are unlikely to cross the blood-brain barrier (BBB).[11] We have identified and synthesized highly specific small-molecule Gal-3 ligands that modulate Gal-3 interactions with TREM2 and affect the inflammatory response in microglial and macrophage cell lines. In vitro characterization and optimization of lead compounds is ongoing. Biodistribution, PK, PD and proof-of-efficacy studies are planned.

Hypothesis

Modulation of the innate immune response towards the homeostatic, neuroprotective state would prove beneficial in ND. Small-molecule drugs that cross the BBB and modulate Gal-3–TREM2 interactions could be an effective anti-inflammatory therapy.

Methods and Results

In silico screening to identify novel Gal-3 ligands

We identified a site on Gal-3 that sterically interferes with CRD binding. Interactions between the N-terminal domain (AA 91-113) and the carbohydrate recognition domain (CRD) (AA 114-245) affect the CRD interactive capacity. We used this novel allosteric site and information on glycan chemistry and lectin interactions for computational analysis and in silico design, screening for organic, non-carbohydrate heterocyclic molecules (**figure 1**). Over 600 compounds were evaluated; 60 proprietary compounds were synthesized and tested, using a fluorescence polarization assay, and a functional ELISA that measured inhibition or enhancement of Gal-3 binding to multiple receptors. Several polyaromatic allosteric compounds fall within the desired parameters for a CNS-targeted drug. Preliminary ADME data indicate stability in human plasma and high permeability in Caco-2 cells. In addition, they are not likely substrates of the P-gp efflux mechanism (**table 1**).

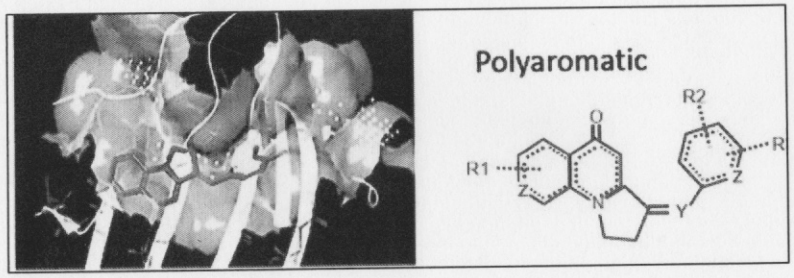

Figure 1. The allosteric binding site on Gal-3 used to design small polyaromatic ligands. Two scaffolds were identified and used for the in-silico design and screening.

Table 1. Physical properties and preliminary ADME data.

compound	MW (Da)	CLogP	HBD	tPSA (Å)	pKa	human plasma stability $t_{1/2}$ (min)	Caco-2 permeability mean A→B P_{app} (10^{-6} cm s^{-1})[a]	mean B→A P_{app} (10^{-6} cm s^{-1})[a]	efflux ratio[b]
G229	292.30	3.43	0	32.67	N/A	439	27.3	24.8	0.91
G164	354.41	5.27	1	60.55	9.2	334	20.0	5.66	0.28

[a] P_{app} - apparent permeability rate coefficient
[b] efflux ratio – P_{app} (B→A) / P_{app} (A→B)

Screening for Gal-3 specificity

Compounds were screened for Gal-3 specificity using a functional ELISA which assessed inhibition of galectin binding to surface-immobilized integrin αMβ2. An ELISA plate was coated with integrin αMβ2, then incubated with galectins, with or without the compounds. Anti-galectin antibodies were used to detect the amount of bound galectin. The compounds were tested against galectins 1, 3, 8 and 9. Approximately 40 compounds selectively blocked Gal-3 binding, compared to TD139, a modified disaccharide Gal-3 inhibitor, which inhibits binding of multiple galectins (**figure 2**).

Modulation of Gal-3 interactions with TREM2

As Gal-3 is an endogenous ligand for the microglial receptor TREM2 and is involved in multiple inflammatory pathologies, we tested the effects of the compounds on this interaction. An ELISA plate was coated with TREM2, then treated with Gal-3, with or without the compounds. An anti-Gal-3 antibody was used to detect bound Gal-3. Compound G164 inhibits Gal-3 binding to TREM2 (**figure 3A**). Compound G929 enhances the interaction between Gal-3 and TREM2 (**figure 3B**).

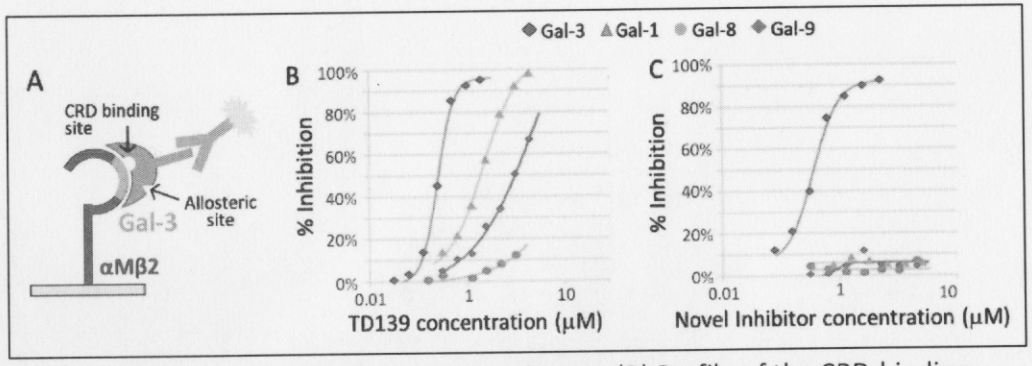

Figure 2. (**A**) Principle of the receptor-based assay. (**B**) Profile of the CRD-binding inhibitor TD139. (**C**) Improved specificity of our allosteric compound G229.

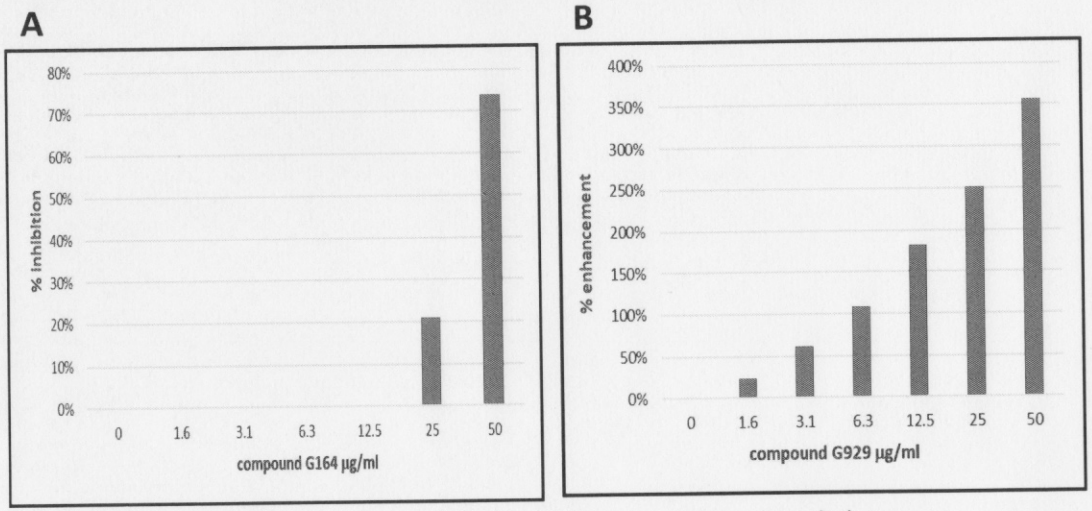

Figure 3. (**A**) Compound G164 inhibits Gal-3 binding to the microglial receptor TREM2. (**B**) Compound G929 increases Gal-3 – TREM2 interaction.

Conclusions and future directions

Preliminary studies show that our small-molecule galectin-3 modulating compounds show high specificity for Gal-3, alter Gal-3 interactions with microglial receptors, inhibit production of the inflammatory cytokine MCP-1 (data not shown) and are predicted to cross the blood-brain barrier. These novel Gal-3 ligands could be a highly effective anti-inflammatory treatment for several neurodegenerative diseases by modulating microglial activation towards neuroprotection. Further in vitro characterization of the compounds will be done, to assess the effects on microglial phenotype and function. Additional ADME studies will be performed as well as optimization to improve solubility. Future biodistribution, PK and PD studies will provide preclinical data on the distribution, stability, and clearance of the compounds. Proof-of-efficacy studies will be done in mouse models of neurodegenerative diseases, including Alzheimer's disease and amyotrophic lateral sclerosis.

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