SMALL-MOLECULE STRUCTURALLY TARGETED ALLOSTERIC REGULATORS OF GLUCOCEREBROSIDASE SHOW NEUROPROTECTIVE PROPERTIES IN CELL-BASED MODELS OF ALZHEIMER'S DISEASE

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Objective

To investigate the potential of structurally targeted allosteric regulators (STARs), also called GT-compounds, to protect against neuronal cell death caused by Amyloid Beta 1-42 (Aβ-1-42) and human Tau oligomers (hTauO) in two separate cell-based models of Alzheimer's disease (AD).

Background

Glucocerebrosidase (GCase) protein levels and enzyme activity are decreased in sporadic AD and overexpression of GCase promotes lysosomal degradation of Aβ1-42 *(Choi et al., 2015)*. Augmentation of GCase activity may therefore be a potential therapeutic option for the treatment of AD. Gain Therapeutics has applied its innovative proprietary drug discovery platform, Site-directed Enzyme Enhancement Therapy (SEE-Tx®), to the discovery and development of orally bioavailable and brain penetrant small-molecule structurally targeted allosteric regulators (STARs) that stabilize GCase by binding to an allosteric site, facilitating its correct folding, maturation and trafficking to the lysosome.

We have shown previously that STAR-mediated augmentation of lysosomal GCase induces a neuroprotective effect in cellular and *in vivo* models of GBA1 Parkinson's disease. Here, we have evaluated the neuroprotective properties of STAR compounds in cellular models of AD: primary rat cortical and hippocampal neurons challenged with Aβ-1-42 and hTauO, respectively.

Misfolded GCase leads to tissue damage and disease development

Misfolded GCase causes ER stress and tissue damage, and is targeted for degradation by the protein quality control system in the ER

GCase protein levels in lysosomes are not sufficient to eliminate toxic substrates (e.g. GlcCer), which leads to build-up of phosphorylated and aggregated α-synuclein

Accumulation of toxic substrates and α-synuclein is associated with cell death, including death of dopaminergic neurons, decreased production of dopamine and increased risk of PD
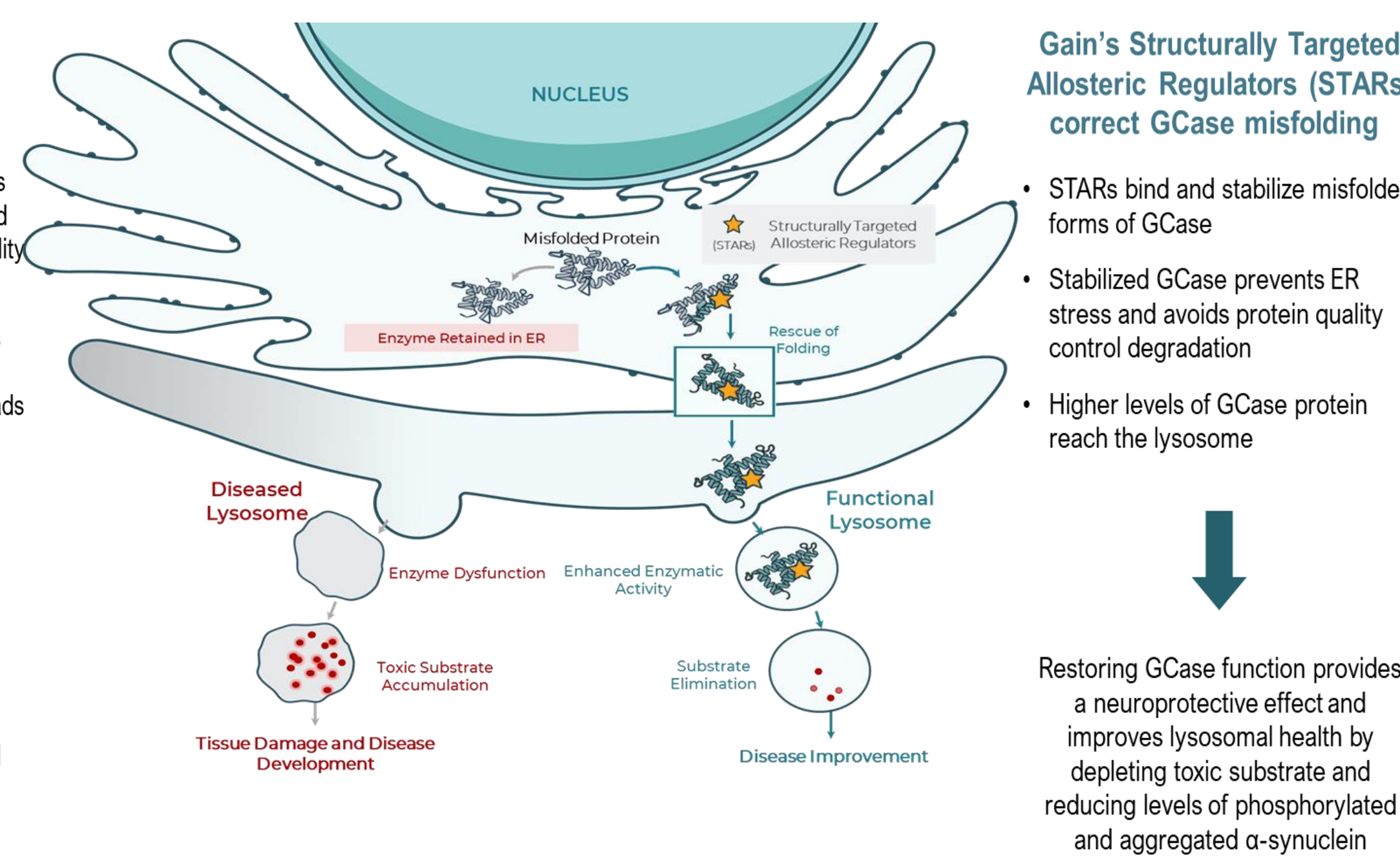
Gain's Structurally Targeted Allosteric Regulators (STARs) correct GCase misfolding

• STARs bind and stabilize misfolded forms of GCase
• Stabilized GCase prevents ER stress and avoids protein quality control degradation
• Higher levels of GCase protein reach the lysosome

Restoring GCase function provides a neuroprotective effect and improves lysosomal health by depleting toxic substrate and reducing levels of phosphorylated and aggregated α-synuclein

Methods

For the Aβ-1-42 assay, primary cortical neurons were prepared from E15 Wistar rat brains. At 7DIV, neurons were pre-incubated with STAR compound B for 96 h and then challenged with Aβ-1-42 (agitated for 3 days at 37°C in the dark) for 24 h at a concentration of 15 μM. Neuronal survival and neurite network were assessed by MAP2 immunostaining and quantification (at 12 DIV). For hyperphosphorylated Tau measurement, pre-incubation with STAR compound B occurred for 48 h and it was assessed by AT-100 immunostaining and quantification (at 12 DIV).

For the hTauO assay, primary hippocampal neurons were prepared from E17 Wistar rat brains. At 6DIV, neurons were pre-incubated with STAR compounds A-C for 96 h and then challenged for 24 h with hTauO at a concentration of 5 μM, prepared from recombinant human tau monomers. Cell viability was investigated using the MTT assay at 24 h post hTauO challenge (11 DIV).

Conclusion

We have demonstrated that our orally bioavailable, brain penetrant STAR compounds show promising activity against Aβ-1-42 and oligomeric Tau toxicity, which are thought to underlie neurodegeneration and cognitive impairment in Alzheimer's disease.

Therefore, STAR therapy emerges as a potential disease-modifying, novel pharmacological option for the treatment of Alzheimer's disease and other tauopathies that warrants further investigation.

References
Choi S et al,.. Lysosomal Enzyme Glucocerebrosidase Protects against Aβ1-42 Oligomer-Induced Neurotoxicity. PLoS One. 2015 Dec 2;10(12):e0143854. doi: 10.1371/journal.pone.0143854.

Results

We report *in vitro* evidence that a STAR compound improves neuronal survival and neurite network and reduces hyperphosphorylated tau in primary rat cortical neurons after Aβ-1-42 injury. Additionally, STAR compounds were shown to reduce hTauO-induced neurotoxicity in primary rat hippocampal neurons.

STAR compound reduces Aβ-1-42-induced neurotoxicity and Tau hyperphosphorylation in rat cortical neurons
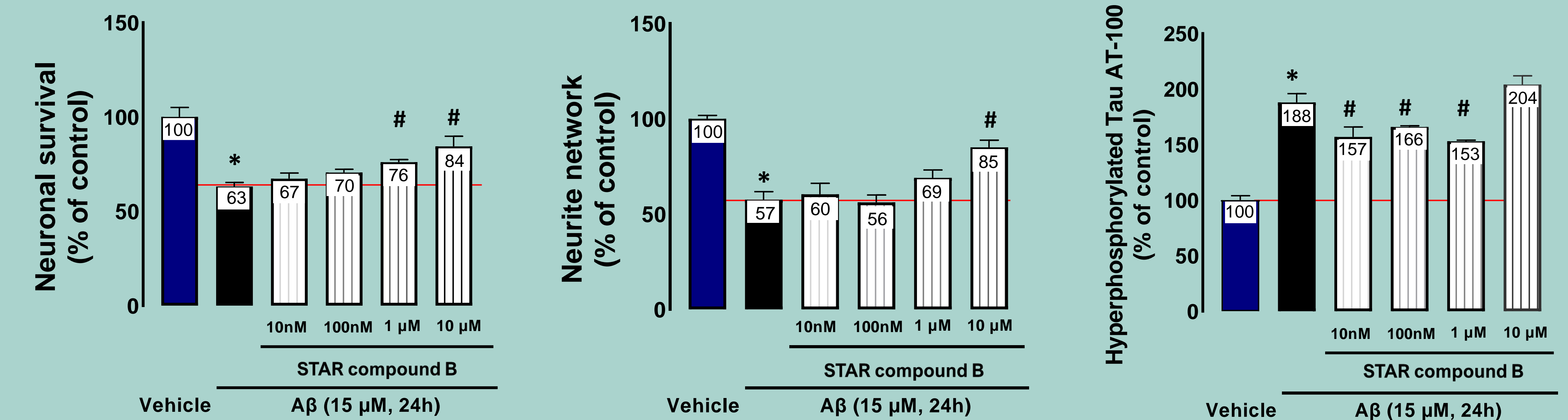

Fig.1 Neuronal survival and neurite network (assessed by MAP2 immunostaining and quantification), and hyperphosphorylation of Tau (assessed by AT-100 immunostaining and quantification) in control neurons (dark blue bars), neurons injured with Aβ 1-42 (black bars) and neurons injured with Aβ 1-42 and treated with the STAR compound B at the following doses: 10 nM, 100 nM, 1 μM and 10 μM (white bars with black lines). Data are expressed as percent of vehicle (set at 100%) and represent the mean ± SEM. * P<0.05 vs vehicle-treated cells, # P<0.05 vs Aβ-1-42-treated cells (One-way ANOVA followed by Fisher's LSD test).

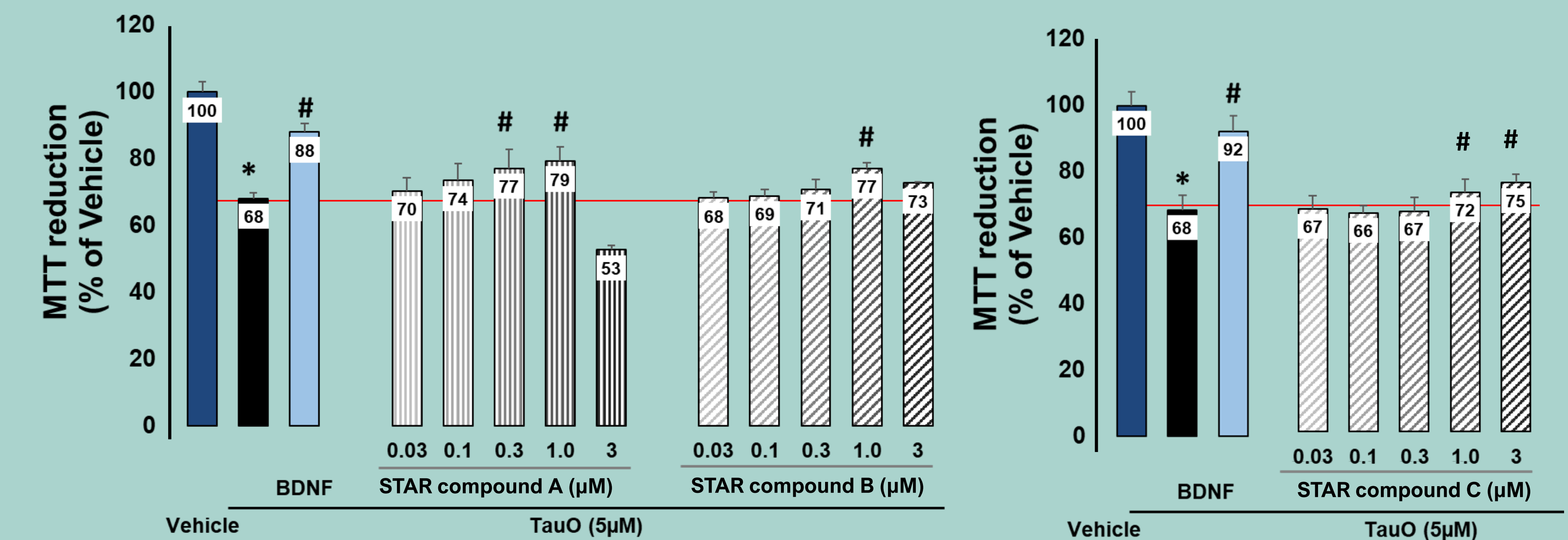### STAR compounds reduce hTauO-induced neurotoxicity in rat hippocampal neurons

Fig.2 Neuronal survival assessed by the MTT assay in control neurons (dark blue bars), neurons injured with hTauO (black bars), neurons injured with hTauO and treated with BDNF (light blue bars) and neurons injured with hTauO and treated with STAR compounds A, B, C and D at the following doses: 0.03 μM, 0.1 μM, 0.3 μM, 1 μM and 3 μM (white bars with black lines). Data are expressed as percent of vehicle (set at 100%) and represent the mean ± SD. * P<0.05 vs vehicle-treated cells, # P<0.05 vs hTauO-treated cells (One-way ANOVA followed by Scheffé's test).