Studies on the use of glycosaminoglycans for the treatment of Alzheimer's disease

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DECLARATION OF ORIGINALITY

This thesis contains no material that has been accepted for the award of any other degree or diploma in any other tertiary institution.

To the best of my knowledge and belief, this thesis contains no material previously published or written by any other person except where due reference is made in the text of the thesis

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Table of Contents

Abstract

Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disorder that is commonly found in the elderly population. AD is characterized pathologically by the deposition of amyloid plaques and neurofibrillary tangles in the brain. The major component of amyloid plaque is the β-amyloid protein (Aβ), a 40-42 amino-acid residue polypeptide that is generated from the β-amyloid precursor protein (APP) by the β-site APP cleaving enzyme-1 (BACE1) and γ-secretase. APP can also be cleaved by α-secretase within the Aβ sequence to form sAPPα and C83, which thus precludes formation of Aβ.

Advances in AD research over the past three decades have not yet led to effective treatments to prevent or cure AD. Therefore, an effective drug for the treatment of AD is required. As oligomeric forms of Aβ are thought to be the major toxic species which cause AD, therapeutic approaches are now targeting the production, clearance or neurotoxicity of Aβ.

It has been reported that glycosaminoglycans (GAGs) such as heparin can influence Aβ production by disrupting APP proteolytic processing. Studies have reported that heparan sulfate and heparin can directly inhibit BACE1 activity in vitro and thereby decrease Aβ production in cell culture. Studies have also shown that heparin binds close to the prodomain of the BACE1 zymogen (proBACE1) and that this binding

stimulates proBACE1 activity. However, heparin can also inhibit mature BACE1 activity by binding close to the active site domain of the mature protein. In contrast, other groups have reported that heparin stimulates β-secretase cleavage of APP in a cultured cell line.

As there are conflicting reports on the effect of GAGs on APP processing and Aβ production, the effects of heparin or enoxaparin on APP processing was first examined in primary cortical cells obtained from transgenic mice expressing human APP_{695} with the Swedish familial AD mutant (Tg2576 mouse). The results showed that heparin or enoxaparin (ENO) treatment can lower Aβ secretion from cortical cells by decreasing BACE1 and thereby inhibiting β-secretase processing of APP. Additionally, treatment with heparin or enoxaparin decreased the α-secretase ADAM10 and inhibited α-secretase processing of APP.

The development of GAG analogues which can be used for the treatment of AD will require the identification of highly potent and specific compounds that have the ability to cross the blood-brain barrier (BBB). Therefore, an aim of the studies in this thesis was to examine the structure specificity (molecular size and sulfation degree) of GAGs with the aim of identifying more potent and specific GAG-based compounds to inhibit APP processing and Aβ production. The effects of various GAGs and sulfated polysaccharides on APP processing were tested in primary cortical cells derived from Tg2576 mice. The results showed that the effect of GAGs on APP processing was both size- and sulfation-dependent. Mucosal heparins (MHs) with small sizes (5 kDa and 3kDa) were less potent in reducing Aβ than high molecular weight MHs (6 kDa and 12.5 kDa). 6-O-Sulfation was important for the effect on APP processing as

heparin lacking 6-O sulfate were less potent than native heparin. However, deletion of carboxyl groups on MH had no significant effect on APP processing. These data suggest that it might be possible to alter the structure of GAGs to achieve more potent and specific inhibitors of APP processing that can cross the blood-brain barrier.

It has been reported that peripheral administration of ENO can reduce the level of Aβ and the amyloid plaque load in the brain of APP transgenic mice. However, the exact mechanism of these effects has been unclear. Therefore, an aim of this study was to examine whether the reduced amyloid plaque load reported to occur in the brains of the APP transgenic mice treated with ENO was due to decreased APP processing to Aβ caused by ENO treatment. ENO was peripherally injected to Tg2576 mice, and the APP processing products and amyloid load in the brains of the mice were examined. The study found that ENO treatment decreased the Aβ40/Aβ42 ratio in cortex and increased the amyloid plaque load in both cortex and hippocampus, while overall APP processing was not significantly influenced by ENO. The exact mechanism of these effects remains unknown. These results suggest that the strategy of using ENO for the treatment of AD may need further assessment.

As GAGs such as HS are widely expressed in the brain in the form of proteoglycans, it is possible that the endogenous HS may also affect APP processing. Therefore, an aim of the study was to examine the role of endogenous HS on APP processing and Aβ production. To examine this, primary cortical cells derived from Tg2576 mice were incubated with a drug or enzyme designed to degrade HS chains from endogenous proteoglycans. The results showed that deletion of endogenous HS can

reduce the level of BACE1 and ADAM10 and thus inhibit APP processing through β- and α-secretase cleavage pathways similar to exogenous treatment of heparin. These findings suggest that regulation of endogenous HS to inhibit APP processing to Aβ could be a novel approach for the treatment of AD.

Based on these results, modification of structures of GAGs or sulfate polysaccharides may achieve highly potent and specific BBB-permeable compounds which can inhibit APP processing to Aβ. Moreover, regulation of endogenous HS can also affect APP processing and Aβ production. Therefore, the studies reported in this thesis support the view that GAG-based compounds can regulate the Aβ production and strategies based on administration of GAGs or the alteration of endogenous GAG metabolism may have value for the treatment of AD.