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Low molecular weight glycosaminoglycan blockade of β-amyloid induced neuropathology

Mark Walzer, Stanley Lorens, Matthew Hejna, Jawed Fareed, Israel Hanin, Umberto Cornelli, John M. Lee*

Departments of Pharmacology and Pathology, Loyola University Chicago Medical Center, 2160 South First Avenue, Rm. 2638, Maywood, IL 60153, USA

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Abstract

Previous studies have shown different roles for proteoglycans and glycosaminoglycans (GAGs) in Alzheimer's disease (AD) neuropathology. Using a rat model of β-amyloid induced neuropathology, we tested whether low molecular weight glycosaminoglycans (Certoparin and C6) could be useful as preventative agents and/or as a potential therapeutic treatment for AD. Chronic subcutaneous low molecular weight glycosaminoglycan injections beginning either before or after an intra-amygdaloid β-amyloid-(25–35) injection blocked abnormal intracellular tau changes and reactive astrocytosis but did not affect β-amyloid's aggregation state. Also, low molecular weight glycosaminoglycan injections beginning 1 day prior to sacrifice did not block the effects of β-amyloid nor did injections of a disaccharide, suggesting chronic low molecular weight glycosaminoglycan treatment is needed to block the effects of β-amyloid. Furthermore, these data indicate that there is a molecular weight range of active low molecular weight glycosaminoglycans in this model; and supports the investigation of low molecular weight glycosaminoglycans as a preventative and/or therapeutic treatment of β-amyloid induced neuropathology. © 2002 Published by Elsevier Science B.V.

Keywords: Alzheimer's disease; Heparin; β-amyloid; Certoparin; Tau; Glycosaminoglycan

1. Introduction

Alzheimer's disease (AD) is characterized histopathologically by senile plaques, neurofibrillary tangles, reactive astrocytosis and regional specific cell loss. Senile plaques are an extracellular deposition of β-amyloid, a 40–42 amino acid peptide, while intracellular neurofibrillary tangles consist of paired helical filaments formed from microtubule associated tau protein in a hyperphosphorylated and/or conformationally altered state (Lang and Otvos, 1992; Watanabe et al., 1992). Previous studies have shown that other components, specifically proteoglycans [heparan sulfate, chrondroitin sulfate, dermatan sulfate, and keratan sulfate] and their glycosaminoglycan (GAG) side chains are co-localized with β-amyloid in senile plaques (Perlmutter et al., 1990; Snow et al., 1987, 1988, 1990, 1994b; Su et al., 1992) and hyperphosphorylated tau in neurofibrillary tangles (DeWitt et al., 1993; Goedert et al., 1996; Perry et al., 1993). In vitro work has shown that proteoglycans and glycosaminoglycans directly bind β-amyloid and synthetic β-amyloid peptides (Brunden et al., 1993; Buee et al., 1993; Fraser et al., 2001; Leveugle et al., 1994; McLaurin et al., 1999b), stimulating fibril nucleation, growth, and stability (Castillo et al., 1997; McLaurin et al., 1999a,b). These data suggest that proteoglycans and their glycosaminoglycan side chains may act as "seed molecules" (Castillo et al., 1997) for β-amyloid fibril formation; hence, promoting senile plaque formation seen in Alzheimer's disease. Much of the research suggests that both charge and size of these proteoglycans/glycosaminoglycans influence their ability to interact with β-amyloid (Castillo et al., 1997, 1998, 1999; Fukuchi et al., 1998).

On the other hand, in vivo studies have shown that glycosaminoglycans and other synthetic polysulfated compounds can attenuate the neurotoxic effects of β-amyloid in cell culture (Pollack et al., 1995a,b; Sadler et al., 1995a,b; Woods et al., 1995) by inhibiting β-amyloid's interaction with cells (Sadler et al., 1995b). Snow et al. (1994a) have shown that when β-amyloid, along with heparan sulfate proteoglycan, is infused into the hippocampus of rats, there

* Corresponding author. Tel.: +1-708-216-8780; fax: +1-708-216-6735.
E-mail address: jlee2@lumc.edu (J.M. Lee).

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is an increased fibrillary β-amyloid deposition in the neuropil. However, when β-amyloid and only the heparan sulfate glycosaminoglycan side chains were co-infused, no Congo-red-positive deposit was detected suggesting alternative roles for proteoglycans and glycosaminoglycans in β-amyloid aggregation in vivo. Castillo et al. (1999) found the presence of glycosaminoglycan sulfate moieties to be critical in the enhancement of β-amyloid fibril formation, as unsulfated heparin glycosaminoglycan did not lead to β-amyloid fibrillogenesis; thus, showing the importance of these glycosaminoglycan sulfate moieties in vivo.

Additionally, clinical studies in Europe have shown a therapeutic effect of Ateroid (a compound made of both high and low molecular weight glycans) on Alzheimer's disease and multi-infarct dementia in elderly patients (Ban et al., 1991; Conti et al., 1989a,b; Cornelli, 1996). Ateroid has also been shown to improve both behavioral and neurochemical impairments seen in aged F344 rats (Lorens et al., 1991) that, along with the clinical studies, suggests a role for glycosaminoglycans in treating not only Alzheimer's disease but also other age-related dementias.

Since Ateroid is a heterogeneous mixture of both high and low molecular weight glycans, an important question is what are the active components of Ateroid? The blood–brain barrier likely prevents the high molecular weight glycan component of Ateroid from entering the central nervous system. However, studies show that low molecular weight glycosaminoglycans are indeed capable of passing the blood–brain barrier (Ma et al., 2000); suggesting that low molecular weight glycosaminoglycans may be the active component of Ateroid.

Based on these studies, we tested whether subcutaneously administered low molecular weight glycosaminoglycans could block the cellular effects of β-amyloid-(25–35) in a rat model of β-amyloid induced neuropathology (Sigurdsson et al., 1996, 1997b). We have also shown that when F344 rats are given an intra-amygdaloid β-amyloid-(1–42) injection, this sequence of β-amyloid induces the same neuropathology as in rats given an intra-amygdaloid β-amyloid-(25–35) injection. This is further supported by findings that an β-amyloid-(25–35) intracerebral ventricular infusion in rats can induce significant short- and long-term memory deficits similar to those seen following a β-amyloid-(1–40) or β-amyloid-(1–42) intracerebral ventricular infusion (Olariu et al., 2001). In the present experiment, animals were given twice a day subcutaneous injections of Certoparin (MW 4600), or a Certoparin derivative (C6; MW 1600) starting either 3 days before, 1 day post, or 8 days post a β-amyloid-(25–35) intra-amygdaloid injection, and continuing for 32 days post β-amyloid injection. This model shows β-amyloid induced neuropathology in the right (ipsilateral) amygdala (injection site) and also in the hippocampus, which sends projections to the amygdala (Sigurdsson et al., 1996) and is one of the earliest brain areas affected in Alzheimer's disease. Hippocampal effects are seen more intensely and consistently in the right hippocampus compared to the left (Sigurdsson et al., 1997b) or amygdala; therefore, this study focused on the effects of β-amyloid in the right hippocampus. Studied endpoints included β-amyloid's β-pleated sheet conformation at the injection site in the amygdala, Tau-2 immunoreactivity and reactive astrocytosis in the CA1, CA2, and CA3 hippocampal regions. This experimental design allowed us to determine whether low molecular weight glycosaminoglycans may be beneficial in β-amyloid induced neuropathology either before and/or after β-amyloid deposition.

2. Materials and methods

The experimental methods have been described previously in detail by Sigurdsson et al. (1996).

2.1. Animals

Male Fischer 344 rats were obtained from Harlan Sprague–Dawley, (Indianapolis, IN). At the time of arrival, the rats weighed 250–300 g and were 3 months of age, had access to food and water ad libitum, and were habituated to their new environment for 2 weeks prior to surgery.

2.2. Surgery

Surgery was performed under sodium pentobarbital (50 mg/kg, i.p.; Butler, Columbus, OH) anesthesia. The animals received a unilateral injection of β-amyloid-(25–35) (5.0 nmol/3 μl) into the right amygdala. A Kopf stereotaxic instrument was used with the incisor bar set at 3.3 mm below the interaural line. Injection coordinates measured from bregma and the surface of the skull (AP −3.0, ML −4.6, DV −8.8) have been empirically determined based on the atlas of Paxinos and Watson (1986). A volume of 3.0 μl was administered over 6 min (flow rate 0.5 μl/min) using a CMA/100 microsyringe pump (Carnegie Medicin, Solna, Sweden). The cannula was left in situ for 2 min following injection and then slowly withdrawn.

2.3. Drugs

(a) β-amyloid-(25–35) (BACHEM, Torrance, CA) is supplied as a trifluoroacetic acid salt with the peptide content of 88% (±3%). The peptide and its respective vehicle (trifluoroacetic acid sodium salt; Sigma) was dissolved in Nanopure® H_2O (dH_2O) immediately before use and stored at 4 °C between injections.

(b) Low molecular weight glycosaminoglycans: Certoparin (Novartis, Switzerland), C6 (Novartis), and a disaccharide were provided by Dr. J. Fareed (Loyola University Medical Center, Maywood, IL). The molecular weights of these compounds were determined by high-performance size-exclusion chromatography (Ahsan et al., 1995) or mass spectrometry, and are approximately 4600, 1600 and 820

Da, respectively. The low molecular weight heparin Certoparin is mainly composed of hexa-decasaccharide units and is clinically used in Germany for the prophylaxis of post-surgical deep vein thrombosis (Fareed et al., 2000). Certoparin is produced by deamination cleavage of unfractionated heparin by isoamyl nitrite digestion, leading to the formation of a 2,5-anhydro-D-mannose (5-member ring) at the reducing and non-reducing ends of the heparin chains (Jeske et al., 1999). Certoparin is heterogeneously composed of 6–10 2-*O*-SO_3-uronic acid-6-*O*-SO_3-glucosamine-*N*-SO_4 disaccharide repeating units with a sulfate/carboxyl ratio of 2.5, equal to that of unfractionated heparin (Jeske et al., 1999). Further isoamyl nitrite digestion of Certoparin and subsequent isolation of the low molecular weight tetra-hexasaccharides products produces the Certoparin derivative C6, while the disaccharide is the disaccharide component of Certoparin. All drugs were provided as a white powder and stored in a desiccator at room temperature. All solutions were made fresh before each injection. Twice a day subcutaneous injections were given in the back of the rat using

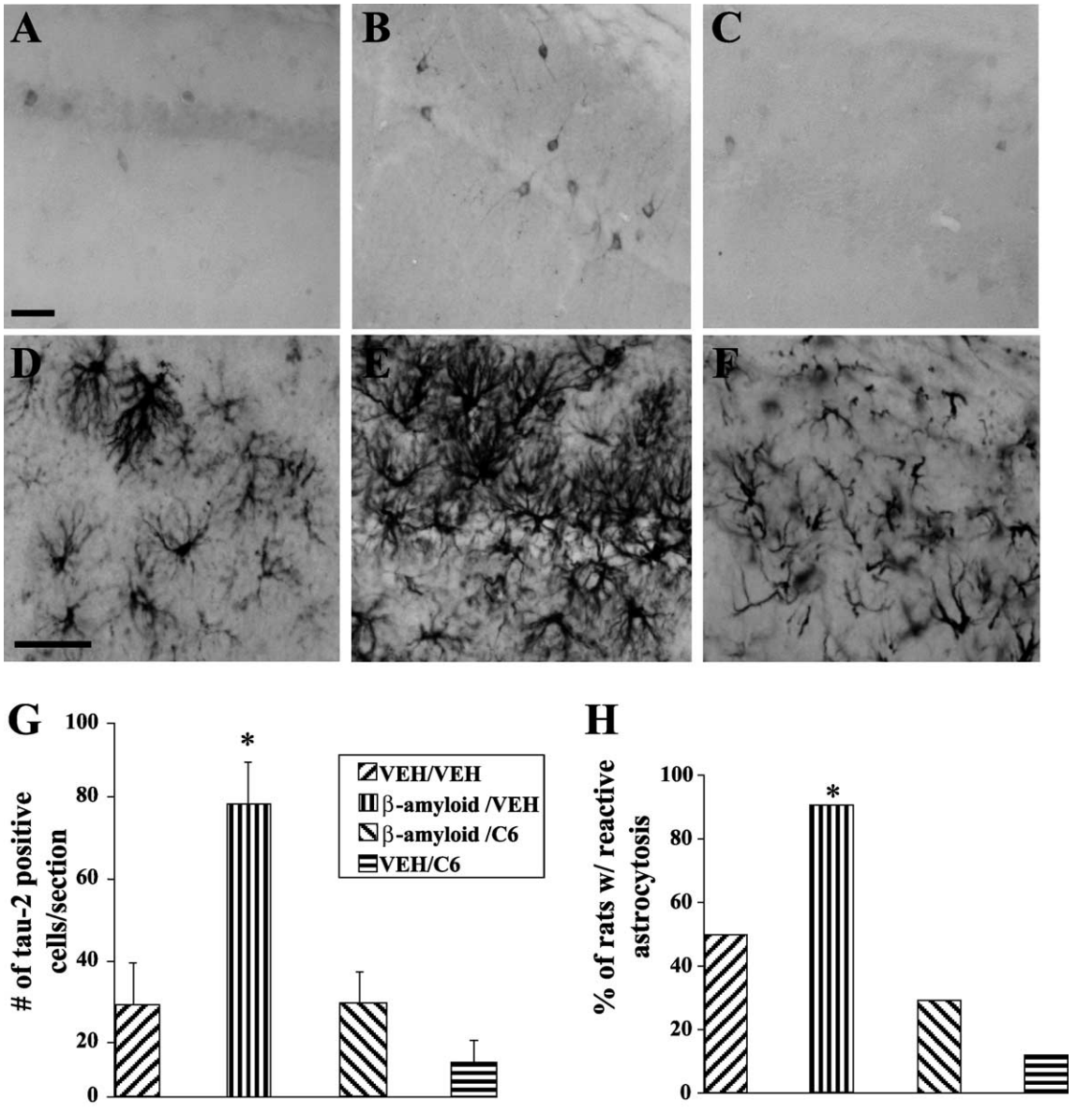

Fig. 1. Treatment effect of chronic low molecular weight glycosaminoglycan (C6) administration beginning 3 days prior to β-amyloid-(25–35) intra-amygdaloid injection. Photomicrographs of coronal rat brain sections showing: (A–C) Tau-2 immunoreactivity in the CA2 hippocampal region of vehicle/vehicle, β-amyloid/vehicle, β-amyloid/C6 animals, respectively (original magnification, ×20); and (D–F) GFAP immunoreactivity in the CA1 hippocampal region of vehicle/vehicle, β-amyloid/vehicle, and β-amyloid/Certoparin animals, respectively (original magnification, ×40). Scale bars (A and D), 50 μm. Graphical analysis of (G) neuronal Tau-2 immunoreactivity within the ipsilateral hippocampus; (H) percentage of rats with reactive astrocytosis as measured by the degree of GFAP immunoreactivity in the ipsilateral hippocampus (based on a 0–2+ rating scale as described in Materials and methods). Each bar represents mean±S.E.M. n=8–12 animals per group. *P<0.05 compared to vehicle/vehicle animals.

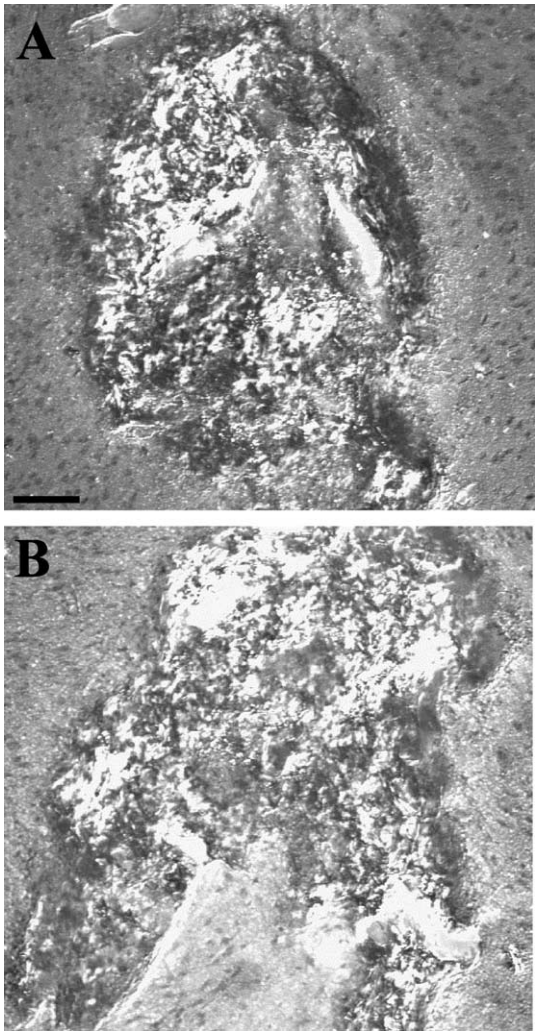

Fig. 2. Congo-red-staining of the ipsilateral amygdala (original magnification, ×20) through the β-amyloid injection site. (A) β-amyloid/vehicle treated rat; and (B) rats given Certoparin treatments beginning 3 days prior to the β-amyloid-(25–35) injection. β-amyloid/vehicle and β-amyloid/low molecular weight glycosaminoglycan-treated animals showed birefringence of the Aβ deposit under polarized light, indicative of a β-pleated sheet structure. Scale bar (A), 100 μm.

a 27 1/2-gauge needle, no observable inflammation occurred over the course of these injections.

2.4. Animal sacrifice and tissue preparation

Animals were anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and perfused transaortically at 32 days postoperatively with 250 ml of 0.1 M sodium/potassium phosphate buffer (PB, pH 7.4) followed by 500 ml of 4% paraformaldehyde in PB at a rate of 500 ml/h at room temperature. Following perfusion the brain was placed in the buffered fixative with 20% sucrose for 1.0 h. The brain was then trimmed into a 6-mm block surrounding the injection site and stored in a solution containing 20% sucrose, 0.1% sodium azide, and 0.01% bacitracin in PB for 24 h at 4 °C. The tissue block was then transferred to a solution containing 20% glycerol and 2% dimethyl sulfoxide dissolved in 0.1 M sodium PB and stored at 4 °C until sectioning. Serial coronal sections (40 μm) were cut on a sliding microtome (American Optical, Buffalo, NY), and five series of sections at 0.2-mm intervals were obtained for histological analysis using the: (1) Congo-red; (2) Tau-2; (3) and glial fibrillary acidic protein (GFAP) immunocytochemistry methods. Sections were then placed in ethylene glycol cryoprotectant and stored at −20 °C until used for immunocytochemistry.

2.5. Immunocytochemistry

The monoclonal Tau-2 antibody (Sigma, St. Louis, MO) is a phosphorylation-independent antibody recognizing both phosphatase and non-phosphatase treated tau (Papasozomenos and Binder, 1987). Additionally, the Tau-2 monoclonal antibody recognizes conformational changes present in neurofibrillary tangles in Alzheimer's disease brains (Lang and Otvos, 1992; Watanabe et al., 1992). However, Tau-2 recognizes Alzheimer's disease tau and not control tau in fixed tissue (Papasozomenos and Binder, 1987). A series of 40-μm sections were removed from cryoprotectant and washed overnight in phosphate-buffered saline (PBS; pH 7.4) at 4 °C. The sections were incubated in Tau-2 monoclonal antibody (Sigma) or GFAP polyclonal antibody (Dako, Carpenteria, CA) at a 1:500 dilution for 24 h at room temperature. Subsequently, the tissue was washed 3 × 10 min in 0.3% Triton X-100 in PBS (PBS-tx) and then incubated for 1.0 h in biotinylated anti-mouse or anti-rabbit immunoglobulin (IgG) secondary antibody (Vectastain ABC Elite kit, Vector Laboratories, Burlingame, CA) diluted in PBS-tx. Following two washes, the tissue was incubated for 1.0 h in avidin horseradish peroxidase (Vector) diluted in PBS-tx. The sections were reacted in 3,3′ diaminobenzidine tetrahydrochloride (DAB) with nickel ammonium sulfate intensification (35 mg DAB, 2.5 g nickel ammonium sulfate per 100 ml sodium acetate buffer with 0.3% H_2O_2 added in 10 μl increments). Subsequently, the tissue was slide mounted, dried, defatted, and cover slipped. According to product specifications, the biotinylated anti-mouse IgG (Vector) has 25% cross-reactivity with rat IgG. Immunolabeled cells in the omit sections were subtracted accordingly to establish a baseline.

Table 1
Comparative molecular weight profile of Certoparin, C6, and disaccharide using high-performance size-exclusion chromatography

Agent	Peak molecular weight	Dispersity[a]
Certoparin	4557	1.68
C6 (Certoparin derivative)	1566	1.17
Disaccharide	820	1.20

According to the method of Ahsan et al. (1995).

[a] Dispersity is a measurement of homogeneity, the closer to 1 the more homogeneous the compound.

2.6. Congo-red

Congo-red staining was done as previously described by Mallory (1961).

2.7. Data analysis

The β-amyloid deposits were analyzed for "apple-green" birefringence (showing β-amyloid in its neurotoxic β-pleated sheet formation) by microscopic examination under polarized light of the Congo-red-stained sections. In all experiments, Tau-2 immunoreactive cells were counted from six coronal sections (40 μm) surrounding the cannula track. The numbers of Tau-2 immunoreactive cells in the ipsilateral hippocampus were analyzed using a one-way analysis of variance (Prism 3.0) followed by a Newman–Keuls' post hoc test. Reactive astrocytosis in the ipsilateral hippocampus was rated on a scale of 0–2+ as previously reported (Sigurdsson et al., 1997b). Reactive astrocytosis was assessed by increases in intensity and arborization of astrocytic processes. An animal was considered positive if it exhibited 1–2+ staining. The GFAP staining was analyzed using a Chi-squared test (Prism 3.0) followed by a Fisher's exact test.

3. Results

3.1. C6 and Certoparin injections beginning 3 days prior to the β-amyloid-(25–35) injection

In order to determine whether low molecular weight glycosaminoglycans inhibit β-amyloid induced neuropathology, we started twice a day subcutaneous injections of saline (vehicle) or 2.5 mg/kg injections of either C6 or Certoparin beginning 3 days prior to an intra-amygdaloid injection of β-amyloid-(25–35). At the 32 day endpoint, we found that the increased Tau-2 immunoreactivity in the ipsilateral hippocampus of β-amyloid/vehicle animals was blocked by injections of C6 ($F[3, 36] = 4.976$, $P<0.001$; Fig. 1A–C, G). Increases in GFAP immunoreactivity found in the right hippocampus of β-amyloid/vehicle animals were also blocked by C6 injections beginning 3 days prior to the Aβ injection ($P<0.05$; Fig. 1D–F, H). Tau-2 analysis was similar when Certoparin was used instead of C6; however, Certoparin administration was unable to decrease β-amyloid induced reactive astrocytosis in these animals. There were no significant differences in Congo-red-staining of the β-amyloid deposits found in β-amyloid/vehicle and β-amy-

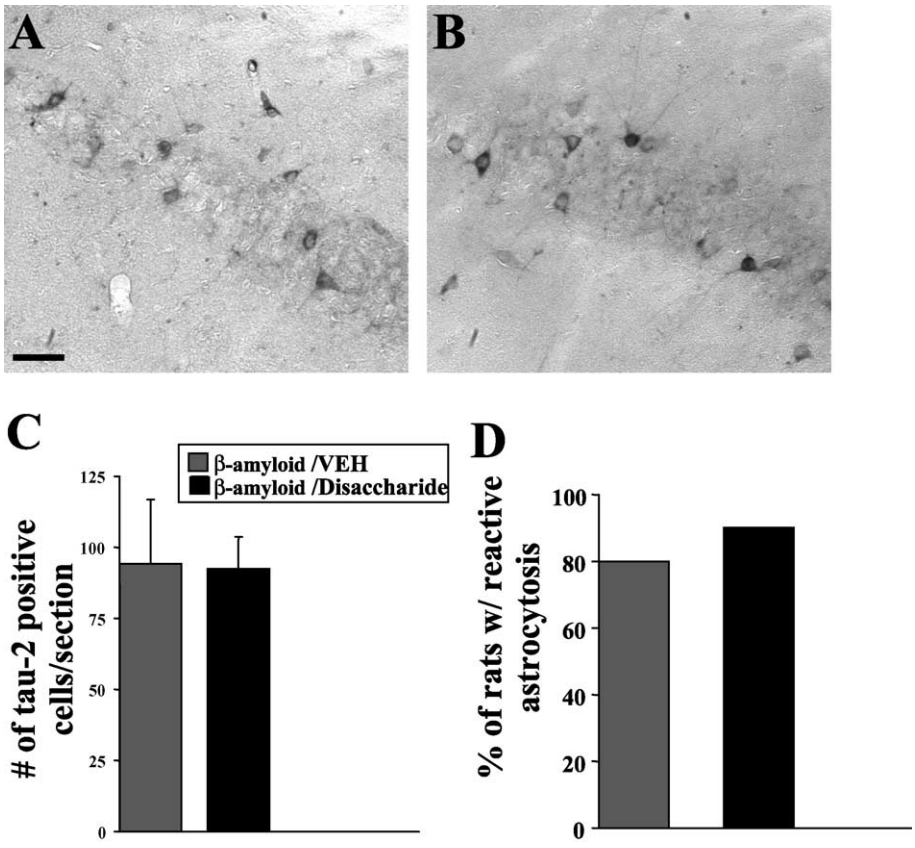

Fig. 3. Disaccharide treatment beginning 3 days prior to β-amyloid-(25–35) intra-amygdaloid injection. Photomicrographs (original magnification, ×20) of Tau-2 immunoreactivity in the ipsilateral hippocampus in (A) β-amyloid/vehicle and (B) β-amyloid/Disaccharide treated animals. Scale bar (A), 100 μm. (C) Neuronal Tau-2 immunoreactivity within the ipsilateral hippocampus; (D) percentage of rats with reactive astrocytosis measured by degree of GFAP immunoreactivity in the ipsilateral hippocampus. Each bar represents mean ± S.E.M. n=8–12 animals per group.

loid/glycosaminoglycan injected animals. Deposits in each treatment group showed apple-green birefringence (Fig. 2). In order to determine whether the varying effects of Certoparin versus C6 on GFAP immunoreactivity were due to differences between the two experiments and not intrinsic differences between the drugs themselves, we repeated the study with both drugs within the same experiment. In this case, both drugs decreased β-amyloid induced Tau-2 and GFAP immunoreactivity, and we found no difference between the effects of Certoparin versus C6 on β-amyloid induced Tau-2 or GFAP immunoreactivity (Walzer et al., 2002).

Based on high-performance size-exclusion chromatography (HPSEC), we determined Certoparin to be a very heterogeneous glycosaminoglycan mixture with extensive molecular weight dispersity as expected based on previous research (Table 1, Jeske et al., 1999). However, the Certoparin derivative, C6, and the disaccharide were both found to be more homogeneous mixtures of glycosaminoglycans as was expected. Therefore, in order to better define the therapeutic molecular size range of these compounds we tested the effectiveness of a disaccharide (MW 820) in this animal model. Subcutaneous injections of the disaccharide (2.5 mg/kg) or saline began 3 days prior to β-amyloid injection and continued for 32 days post β-amyloid injection. Tau-2 ($t=0.1703$, $df=9$, $P=0.348$; Fig. 3A–C) and GFAP immunoreactivity analysis ($P>0.05$; Fig. 3D) revealed no significant effect of the disaccharide on these two end-points.

3.2. C6 and Certoparin injections beginning 1 day after the β-amyloid-(25–35) injection

In vivo and in vitro work has shown that β-amyloid aggregation is an important factor in β-amyloid induced cellular responses (Kowall et al., 1991; Lorenzo and Yankner, 1994; Pike et al., 1993). Therefore, to determine whether low molecular weight glycosaminoglycans could block the effects of aggregated β-amyloid-(25–35), animals were given low molecular weight glycosaminoglycan or vehicle injections beginning 1 day post β-amyloid injection. Certoparin blocked the β-amyloid induced Tau-2 immunoreactivity ($F[3, 33]=6.723$, $P<0.01$; Fig. 4A–D) and reactive astrocytosis ($P<0.05$; Fig. 4E) in the right hippocampus. C6 treatment also blocked β-amyloid induced Tau-2 immunoreactivity in the right hippocampus ($F[3, 34]=23.45$, $P<0.001$). However, C6 administration did not block β-amyloid induced reactive astrocytosis in the right hippocampus when treatment began 1 day after the β-amyloid injection.

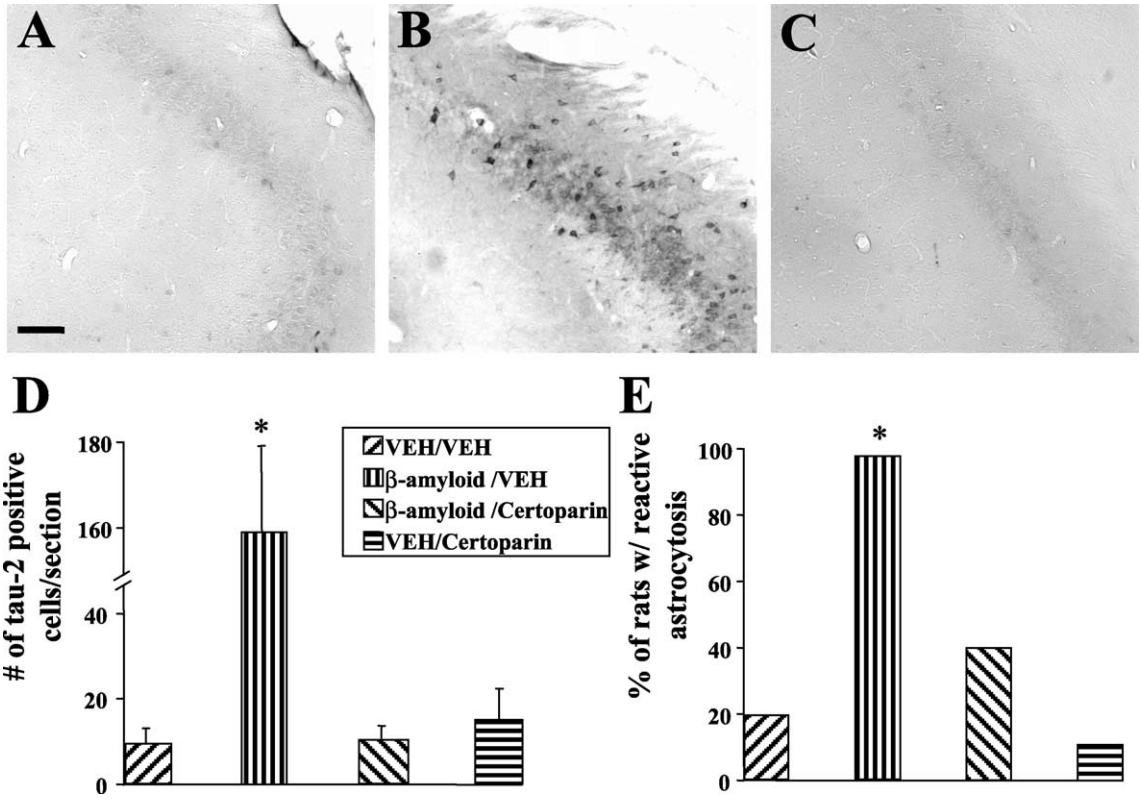

Fig. 4. Treatment effects of chronic Certoparin administration beginning 1 day post β-amyloid-(25–35) intra-amygdaloid injection. Photomicrographs (original magnification, ×10) of coronal rat brain sections showing Tau-2 immunoreactivity in the CA2 hippocampal region of (A) vehicle/vehicle, (B) β-amyloid/vehicle and (C) β-amyloid/Certoparin-treated rats. Scale bar (C), 100 μm. (D) Number of neuronal Tau-2 immunoreactive cells within the ipsilateral hippocampus section; (E) percentage of rats with reactive astrocytosis measured by degree of GFAP immunoreactivity in the ipsilateral hippocampal section. Each bar represents mean±S.E.M. $n=8–12$ animals per group. *$P<0.05$ compared to vehicle/vehicle animals.

3.3. C6 and Certoparin injections beginning 8 days after the β-amyloid-(25–35) injection

Studies by Sigurdsson et al. (1996) showed that β-amyloid-(25–35) increases Tau-2 and GFAP immunoreactivity within 8 days after an intra-amygdaloid injection. Therefore, to test whether low molecular weight glycosaminoglycans could block the effects of β-amyloid even after cellular responses to β-amyloid have been initiated, we started twice a day subcutaneous injections of low molecular weight glycosaminoglycans or saline 8 days after the β-amyloid injection. Tau-2 immunoreactivity (F[3, 34]= 5.241, P<0.01; Fig. 5A) and GFAP immunoreactivity (P<0.05; Fig. 5B) in the right hippocampus was decreased in β-amyloid/Certoparin animals compared to β-amyloid/vehicle treated rats. However, β-amyloid/C6 rats showed only a Tau-2 immunoreactivity decrease (F[3, 31]=8.749, P<0.001) and no decrease in GFAP immunoreactivity (P>0.05) compared to β-amyloid/vehicle treated rats (data not shown).

3.4. C6 injections beginning 31 days after the β-amyloid-(25–35) injection

In order to determine whether the decrease by glycosaminoglycans of β-amyloid induced Alzheimer's disease-like neuropathology was due to long-term treatment (≥24 days) and not an acute phenomenon we gave only two subcutaneous injections of C6 1 day prior to animal sacrifice. Immunohistological analysis revealed no significant decrease of either Tau-2 (P>0.05) or GFAP (P>0.05) immunoreactivity following these two subcutaneous injections of C6 (data not shown).

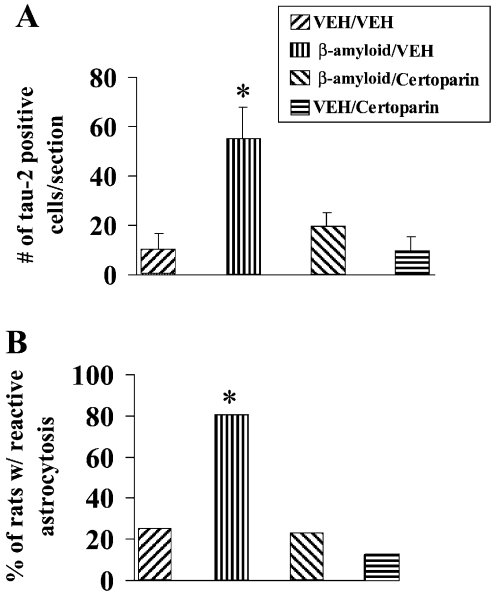

Fig. 5. Treatment effects of chronic Certoparin administration beginning 8 days post β-amyloid-(25–35) intra-amygdaloid injection. (A) Neuronal Tau-2 immunoreactivity within the ipsilateral hippocampus; (B) percentage of rats with reactive astrocytosis measured by degree of GFAP immunoreactivity in the ipsilateral hippocampus. Each bar represents mean ± S.E.M. n=8–12 animals per group. *P<0.05 compared to vehicle/vehicle animals.

4. Discussion

These experiments demonstrate that low molecular weight glycosaminoglycans are able to prevent β-amyloid induced tau conformational changes and reactive astrocytosis if given either before or after β-amyloid deposition has occurred. Additionally, the effects of low molecular weight glycosaminoglycans are independent of β-amyloid deposit formation, as these compounds do not break up the injected β-amyloid deposit in this model.

Previous studies have shown β-amyloid can induce tau phosphorylation and conformation changes (Chambers et al., 2000; Kowall et al., 1992; Sigurdsson et al., 1996, 1997a,b; Takashima et al., 1998) suggesting a link between β-amyloid and altered tau pathology. Gotz et al. (2001) recently showed an intrahippocampal β-amyloid-(1–42) injection could significantly accelerate neurofibrillary tangle formation and reactive astrocytosis in P301L mutant tau transgenic mice (Gotz et al., 2001). Interestingly, both the aforementioned mouse model and our rat model show β-amyloid induced neuropathology at sites distant from the β-amyloid injection site. Supporting the hypothesis that damage to presynaptic terminals projecting to the β-amyloid injection site can lead to altered tau pathology. The present model provides additional support for the role of β-amyloid in inducing tau phosphorylation and conformation changes in Alzheimer's disease. The ability of low molecular weight glycosaminoglycans to prevent these changes provides compelling evidence for their therapeutic effects in Alzheimer's disease.

Recently, we have shown a similar low molecular weight glycosaminoglycan (C3; MW 1900) is capable of crossing the blood–brain barrier if given to rats either intravenously, subcutaneously or orally (Ma et al., 2000). Since Certoparin and C6 are heterogeneous compounds, it is likely that lower molecular weight glycosaminoglycan fractions are crossing the blood–brain barrier causing the effects seen in this study. Currently, it is unknown what molecular weight glycosaminoglycan fraction of these heterogeneous compounds crosses the blood–brain barrier. However, the present data demonstrate that there is a molecular weight range of glycosaminoglycans that block the effects of β-amyloid-(25–35), as the disaccharide was unable to block β-amyloid's cellular effects (Table 2).

The different effects of Certoparin and C6 on reactive astrocytosis in the 1- and 8-day post studies suggest that we may be in the linear range of a dose–response curve. Slight differences in absorption, distribution (specifically the ability to cross the blood–brain barrier) and/or elimination of these two drugs, due to their different molecular weights,

Table 2
Ability of low molecular weight glycosaminoglycans to block β-amyloid induced Tau-2 and/or GFAP immunoreactivity in the right hippocampus of male F344 rats

Injections beginning	Certoparin (MW 4600)		C6 (1600)		Disaccharide (820)	
	Tau-2	GFAP	Tau-2	GFAP	Tau-2	GFAP
3 days prior	+	+	+	+	–	–
1 day post	+	+	+	–	n.t.	n.t.
8 days post	+	+	+	–	n.t.	n.t.
31 days post	n.t.	n.t.	–	–	n.t.	n.t.

"n.t." stands for not tested.

may account for their different effects. Additional dosing and pharmacokinetic studies are needed to investigate this theory.

Based on previous research showing glycosaminoglycans could bind β-amyloid and prevent fibril formation, it was originally hypothesized that low molecular weight glycosaminoglycans may prevent β-amyloid from forming its neurotoxic β-pleated sheet conformation in vivo, subsequently blocking β-amyloid induced neuropathology. However, upon Congo-red-staining analysis, there was no measurable effect of low molecular weight glycosaminoglycan treatment on β-amyloid's β-pleated sheet conformation. We also found that low molecular weight glycosaminoglycans blocked the effects of β-amyloid even if given after the deposit formation and after the initial insult had occurred. These data suggest glycosaminoglycans are able to prevent cellular responses to β-amyloid without altering β-amyloid deposition. Interestingly, Kisilevsky et al. (1995) found that small-molecule disulfates (MW 900–1000) could inhibit the in vitro acceleration of β-amyloid amyloid fibril formation by heparan sulfate. Although these compounds are slightly smaller in molecular mass than Certoparin or C6 they do have a similar amount of sulfate residues substantiating the importance of sulfates in inhibiting β-amyloid induced neuropathology and/or fibril formation. Since there is no measurable change in the Congo-red-stained positive deposit after low molecular weight glycosaminoglycan treatment, it is unlikely that low molecular weight glycosaminoglycans are acting as β-amyloid fibrillogenesis inhibitors. Alternatively, in vitro studies have shown glycosaminoglycans may act by coating β-amyloid thus preventing β-amyloid from interacting with neurons (Sadler et al., 1995b). This mechanism would explain why low molecular weight glycosaminoglycan treatment leads to a decrease of β-amyloid induced neuropathology without altering the Congo-red-stained positive deposit in the present animal model. Further experiments are needed to investigate this mechanism.

Many researchers have shown that the effects of β-amyloid are dependent upon β-amyloid aggregating into its neurotoxic β-pleated sheet conformation. β-amyloid-(25–35) forms fibrils within hours after it is put in solution (Kowall et al., 1992). Hence, we started twice a day low molecular weight glycosaminoglycan injections 1 day following β-amyloid injection to determine if low molecular weight glycosaminoglycan treatment could prevent the cellular effects of aggregated β-amyloid-(25–35). Again, both C6 and Certoparin blocked β-amyloid induced Tau-2 and GFAP immunoreactivity suggesting that low molecular weight glycosaminoglycans can block the cellular effects of β-amyloid after β-amyloid deposition and aggregation.

Earlier characterization of this model identified a significant increase in both Tau-2 and GFAP immunoreactivity as early as 8 days following the β-amyloid injection, showing β-amyloid produces an early cellular response which subsequently peaks at 32 days and returns to basal levels by 96 days (Sigurdsson et al., 1997a). Therefore, low molecular weight glycosaminoglycan treatment was started 8 days after β-amyloid injection to determine whether low molecular weight glycosaminoglycan treatment could block the effects of β-amyloid even after initial cellular responses to the β-amyloid insult have occurred. Interestingly, Certoparin treatment starting 8 days after β-amyloid injection blocked both Tau-2 and GFAP immunoreactivity; however, C6 treatment only blocked Tau-2 immunoreactivity. This suggests that Certoparin treatment may be able to recover early deficits induced by β-amyloid deposition in Alzheimer's disease while C6 administration may be more useful as a preventative agent against β-amyloid induced neuropathology.

To determine whether the effects of low molecular weight glycosaminoglycan administration were due to chronic rather than acute treatment, we gave two subcutaneous C6 injections 31 days after β-amyloid injection (1 day before animal sacrifice). The inability of C6 treatment to block Tau-2 or GFAP immunoreactivity suggests that low molecular weight glycosaminoglycans are not acting through an acute mechanism but rather chronic treatment is needed to block the cellular responses to an β-amyloid insult.

One possible mechanism to explain the effects of low molecular weight glycosaminoglycans in this model is via their role is neurotrophic modulators. Heparin, heparan sulfate and heparin-derived glycosaminoglycan oligosaccarhides have been shown to bind and potentiate the effects of fibroblast growth factors (FGF) (Damon et al., 1988, 1989; Neufeld et al., 1987; Walicke, 1988; Zhou et al., 1997). In addition, recent data suggests that low molecular weight glycosaminoglycan treatment also leads to an increase in both dendritic branching and dendritic spine densities in the present animal model (Mervis et al., 2000). It is possible that heparin-derived Certoparin and C6 may act as modulators of FGF, potentiating FGF induced mitogenic and injury recovery processes, effectively allowing cells to better recover from the β-amyloid insult.

Finally, the present study provides additional support for the further investigation of low molecular weight glycosaminoglycans as a potential therapeutic treatment for β-amyloid induced neuropathology, specifically in Alzheimer's disease.

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