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Involvement of chondroitin 6-sulfation in temporal lobe epilepsy☆

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ABSTRACT

Chondroitin sulfate proteoglycans (CSPGs) are predominant components of the extracellular matrix in the central nervous system (CNS). Previously, we found an increase in the 4-sulfation/6-sulfation (4S/6S) ratio of CSPGs is required for perineuronal net (PNN) formation and results in functional maturation of parvalbumin-expressing interneurons (PV cells) and termination of the critical period in the visual cortex. Here, we report that chondroitin 6-sulfation and chondroitin 6-sulfation-enriched PNNs increased in the mouse cerebral cortex and hippocampus after kainic acid (KA) treatment; simultaneously, chondroitin 4-sulfation-enriched PNNs and the 4S/6S ratio decreased. Furthermore, *chondroitin 6-O-sulfotransferase-1* (*C6ST-1*) transgenic (TG) mice, which overexpress chondroitin 6-sulfated chains and have a decreased 4S/6S ratio, were more susceptible to KA-induced seizures than wild-type mice. These results suggested that chondroitin 6-sulfation is relevant to epilepsy most probably because of dysregulated PNN formation and PV cell maturation.

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1. Introduction

Epilepsy is a very common neurological disorder that presents in a spectrum. Findings from animal models and human postmortem samples indicate that dysfunction of inhibitory GABAergic circuits may be a cause of epilepsy (Powell, 2013; Sgadò et al., 2011). PV cells, which are among the major inhibitory GABAergic interneurons, have an important role in regulation of the inhibitory/excitatory balance in the mammalian brain. In the adult mammalian brain, most of PV cells are enwrapped with PNNs, specialized extracellular matrix structures (Celio et al., 1998). PNNs comprise several CSPGs, hyaluronan, tenascin-R, and cartilage link proteins, and they interdigitate with synaptic contacts (Galtrey and Fawcett, 2007; Powell, 2013). PNN formation begins during late embryonic development and is completed following the critical period (Miyata and Kitagawa, 2015). Several secreted molecules, including Otx2, Sema3A, and Narp, accumulate within PNNs around PV cells; PNNs limit diffusion of these molecules or direct them to bind CS chains, which leads to maturation of PV cells and termination of the critical period (Beurdeley et al., 2012; Chang et al., 2010; Dick et al., 2013).

Each CSPG comprises a core protein with covalently attached chondroitin sulfate (CS) glycosaminoglycan (GAG) chains (Mikami and Kitagawa, 2013), and the functional information of CSPGs may be encoded by the specific sulfation sequence on CS chains (Kitagawa et al., 1997; Miyata et al., 2012). CS chains are long linear polysaccharides composed of repeating disaccharide units; each unit comprises a glucuronic acid and an *N*-acetylgalactosamine (GalNAc) residue. During biosynthesis, individual GalNAc residues of the repeated disaccharide units can be sulfated at C6 or C4 by chondroitin 6-*O*-sulfotransferase-1 (C6ST-1) and chondroitin 4-*O*-sulfotransferase-1 (C4ST-1), respectively (Mikami et al., 2003; Tsutsumi et al., 1998). CSPGs enriched with 4-sulfated GalNAc form conventional PNNs, which can be labeled with *Wisteria floribunda* agglutinin (WFA), around PV cells; these PNNs form a meshwork structure that tightly encases synaptic contacts (Härtig et al., 1992). In contrast, CSPGs enriched with 6-sulfated GalNAcs form abnormal PNNs, which can be labeled with CS56 antibody; these PNNs have a diffusely spread and less condensed morphology (Maeda et al., 2003; Miyata and Kitagawa, 2015). WFA+ PNNs do not colocalize with CS56+ PNNs. A developmental increase in the 4S/6S ratio of CSPGs is required for normal formation of WFA+ PNNs and results in the termination of the critical period for ocular dominance plasticity in the mouse visual cortex (Miyata et al., 2012). Transgenic (TG) mice that overexpress *C6ST-1* retain a low 4S/6S ratio and have fewer WFA+ PNNs and more CS56+ PNNs than control mice. *C6ST-1* TG mice show juvenile-like ocular dominance plasticity in adulthood. Overexpression of C6ST-1 prevents the maturation of electrophysiological properties of PV cells and reduces the inhibitory effects of PV cells because of abnormal PNN formation (Miyata et al., 2012).

PNNs can potentially protect neurons from various toxins, such as neurodegeneration-inducing iron and oxidizing agents (Cabungcal

Abbreviations: CNS, central nervous system; C4ST, chondroitin 4-*O*-sulfotransferase; C6ST, chondroitin 6-*O*-sulfotransferase; CS, chondroitin sulfate; TG, transgenic; PG, proteoglycan; GABA, γ-aminobutyric acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Narp, neuronal activity-regulated pentraxin; Otx2, orthodenticle homeobox 2; PNNs, perineuronal nets; PV cells, parvalbumin-expressing inhibitory neurons; Sema3A, semaphorin3A; WFA, *Wisteria floribunda* agglutinin.

☆ Footnotes: Chondroitin 6-sulfation and epilepsy

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et al., 2013; Suttkus et al., 2014). Dysregulation of PNNs is implicated in various neurological and psychiatric disorders including epilepsy, Alzheimer's disease, bipolar disorder, schizophrenia, and traumatic brain injury (Baig et al., 2005; Berretta et al., in press; McRae and Porter, 2012; Morawski et al., 2010; Okamoto et al., 2003; Pantazopoulos et al., 2015; Yi et al., 2012). Additionally, alteration of C6ST-1 expression and CS sulfation patterns are reportedly evident in brains of human patients with bipolar disorder or schizophrenia and mice with cortical brain injury (Okuda et al., 2014; Pantazopoulos et al., 2015; Yi et al., 2012).

Thus, we hypothesized that sulfation patterns of CS chains are relevant to epilepsy because of PNN formation and PV cell maturation. To test this hypothesis, we examined CS chain sulfation patterns and PNN formation in the cerebral cortex and hippocampus of brains from adult mice that had experienced kainic acid (KA)-induced seizures; we also examined seizure susceptibility of *C6ST-1* TG mice, which overexpress chondroitin 6-sulfated chains.

2. Materials and methods

2.1. Animals

The following mouse lines were used in this study: *C6ST-1* TG mice (Miyata et al., 2012) and C57BL/6J mice. Mice were kept under pathogen-free conditions in an environmentally controlled clean room at the Institute of Laboratory Animals, Kobe Pharmaceutical University. All experiments were conducted according to institutional guidelines regarding ethics for animal experiments and safety for gene manipulation experiments. All animal procedures were approved by the Kobe Pharmaceutical University Committee on Animal Research and Ethics.

2.2. KA treatment and seizure scoring

Mice 8- to 12-weeks old were used in this study. Each mouse was injected intraperitoneally with either saline (vehicle) or 25 mg/kg KA (Wako or Sigma-Aldrich) dissolved in saline and then killed 1 day or 1 week after injection. The KA-treated group was always compared to a time-matched vehicle-treated group. To compare the degree of epileptiform seizures, we recorded the seizure score for 1 h after injection (vehicle or KA) as follows: 0, no reaction; 1, arrest of motion; 2, myoclonic jerks of the head and neck, with brief twitching movements; 3, unilateral clonic activity; 4, bilateral forelimb tonic and clonic activity; 5, generalized tonic–clonic activity with loss of postural tone including death from continuous convulsion; the ratings and criteria were described previously (Yang et al., 1997). Only brains from mice that displayed seizure levels higher than 3 were used as KA-treated samples for digital PCR analysis, disaccharide analysis, and immunohistochemical analysis.

2.3. Digital PCR analysis

Maxwell® 16 Total RNA Purification Kit (Promega) was used according to the manufacturer's protocols to extract total RNA from hippocampus and cortex samples from mouse brains. For reverse

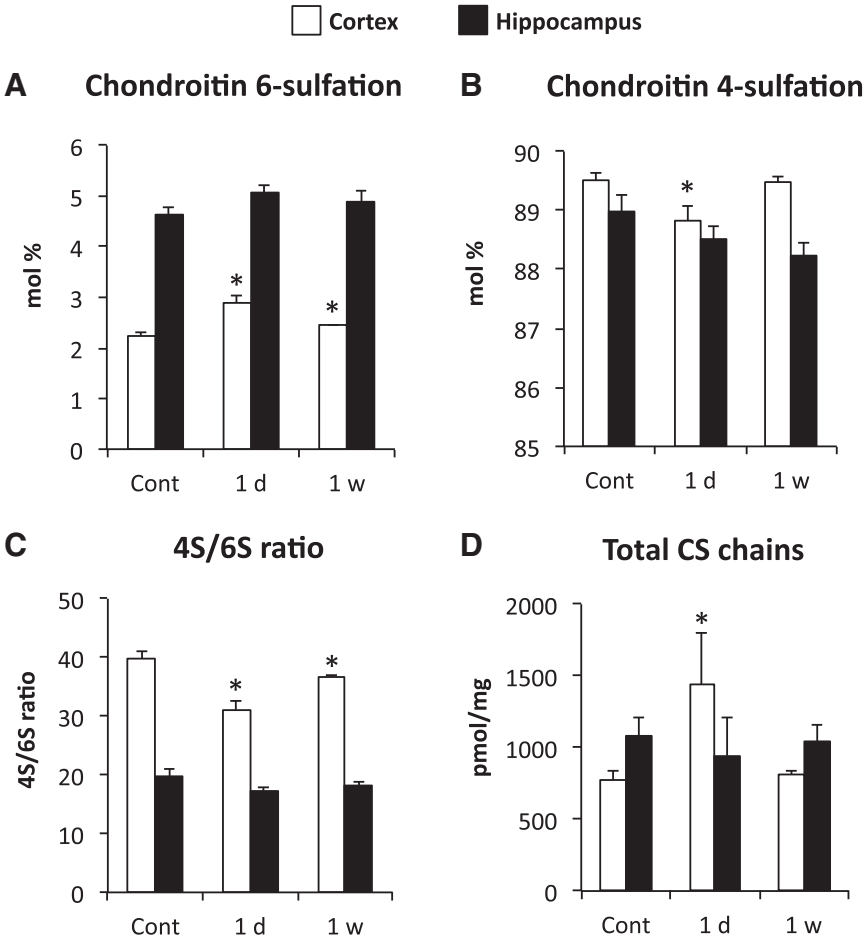

Fig. 1. Sulfation patterns of chondroitin sulfate in wild-type mouse brain after KA treatment. The proportion of chondroitin 6-sulfated (A) and chondroitin 4-sulfated (B), the 4S/6S ratio (C) and the amounts of all CS chains (D) in the cerebral cortex (open bars) and hippocampus (closed bars) of wild-type mice 1 day and 1 week after KA treatment. Molar percentages were calculated by dividing the amount of chondroitin 6- and 4-sulfation individually by the total amounts of disaccharides. The 4S/6S ratio was calculated by dividing the molar percentage of chondroitin 4-sulfation by that of chondroitin 6-sulfation. Error bars represent SEM (n = 3-4 for each group). *$P < 0.05$ (Student's *t* test) between control and each group.

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