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Brief Communication

Structure–activity analysis and antiprion mechanism of isoprenoid compounds

Taichi Hamanaka, Keiko Nishizawa, Yuji Sakasegawa, Kenta Teruya, Katsumi Doh-ura *

Department of Neurochemistry, Tohoku University Graduate School of Medicine, 2-1 Seiryocho, Sendai 980-8575, Japan

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ABSTRACT

The prion strain-specific mechanism by which normal prion protein is converted to abnormal prion protein remains largely unknown. This study found that insect juvenile hormone III reduced abnormal prion protein levels only in cells infected with the RML prion. We conducted a structure–activity analysis using juvenile hormone III biosynthetic intermediates in the isoprenoid pathway. Both farnesol and geranylgeraniol, the most potent inhibitors of abnormal prion protein formation, behaved in an RML prion-dependent fashion. Neither of them modified cellular and cell surface prion protein levels. Events downstream of this pathway include cholesterol biosynthesis and protein prenylation. However, neither of these isoprenoid compounds modified lipid raft microdomains and cellular cholesterol levels and neither affected the representative prenylated protein expression levels of prenylation pathways. Therefore, these isoprenoid compounds are a new class of prion strain-dependent antiprion compounds. They are useful for exploring strain-specific prion biology.

Introduction

Prion diseases are progressive, fatal neurodegenerative illnesses. They include Creutzfeldt–Jakob disease in humans and scrapie, bovine spongiform encephalopathy, and chronic wasting disease in animals. These diseases are characterized by the accumulation of abnormal prion protein (abnormal PrP), the main component of the pathogen, and it is conformationally transformed from the normal prion protein (PrPc) (Prusiner, 1998). Abnormal PrP forms an insoluble protein polymer with a protease-resistant core (PrPres). The conversion of PrPc to abnormal PrP and the turnover of abnormal PrP in prion-infected cells are key events in prion disease, but they remain largely enigmatic.

As remedies for these diseases, dozens of compounds or substances reportedly either inhibit prion formation in prion-infected cultured cells or prolong incubation periods in prion-infected animals (Sim, 2012; Teruya and Doh-ura, 2013; Trevitt and Collinge, 2006). Some are known to be effective in a prion strain-dependent fashion (Berry et al., 2013; Ishikawa et al., 2004, 2006; Kawasaki et al., 2007), but the mechanism underlying prion strain-dependent efficacy remains to be determined. A few antiprion compounds have been used in clinical trials against human prion diseases, but meaningfully beneficial effects in patients have never been reported (Collinge et al., 2009; Geschwind et al., 2013; Haïk et al., 2014; Otto et al., 2004; Tsuboi et al., 2009).

To obtain a starting point for the development of remedies and to elucidate the enigmatic PrP conversion mechanism, we have searched for compounds or substances that modify abnormal PrP formation in prion-infected cells. In earlier studies (Kocisko et al., 2003; Korth et al., 2001), an extensive array of medicinal drugs and biological substances were screened for antiprion activity. We specifically examined untouched materials from natural products. Then we reported antiprion substances extracted from natural products such as fucoidan (Doh-Ura et al., 2007) and protein-bound polysaccharide K (Hamanaka et al., 2011). The insect is a yet under-cultivated natural resource that exists abundantly worldwide in terms of both number and variety. It might be possible to discover new insect-derived substances that are useful either for probing the PrP conversion mechanism or for developing therapeutic and prophylactic treatments for the illness. In fact, anticancer compounds and antivirus compounds have been found in

Abbreviations: PrP, prion protein; PrPc, normal cellular PrP; PrPres, protease-resistant abnormal PrP; JH-III, juvenile hormone III; FOH, farnesol; GGOH, geranylgeraniol

* Correspondence to: Department of Neurochemistry, Tohoku University Graduate School of Medicine 2-1 Seiryocho, Aoba-ku, Sendai 980-8575, Japan. Tel.: (+81)-22-717-8232; Fax: +81 22 717 7656.
E-mail address: doh-ura@med.tohoku.ac.jp (K. Doh-ura).

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insect extracts (Ezzati-Tabrizi et al., 2013; Natori, 1994; Ratcliffe et al., 2011; Slocinska et al., 2008).

For this study, we screened insect-derived substances, such as hormones and biological peptides, using prion-infected cells. Results showed that juvenile hormone III (JH-III) inhibited abnormal PrP formation in cells infected with the RML prion. JH-III is classified as an isoprenoid compound having a farnesane backbone. Because no reports have described the antiprion activity of isoprenoid compounds, we performed a structure–activity analysis of isoprenoid compounds and examined their antiprion mechanism in prion-infected cells. The findings indicate that the antiprion activities of isoprenoid compounds are mediated neither through the modification of PrPc and lipid rafts, nor through the prenylation of proteins. The results of this study suggest that isoprenoid compounds are a new type of probe for elucidating prion strain-dependent abnormal PrP formation.

Results

Antiprion activities of insect-derived substances

Using persistently prion-infected cells, we examined whether insect-derived substances have antiprion activity or not. Sixteen substances, including hormones and their analogues as well as biological peptides, were tested in three distinct prion strain-infected neuroblastoma cell lines: N2a cells infected with RML prion (ScN2a cells), N2a cells infected with 22 L prion (N167 cells),

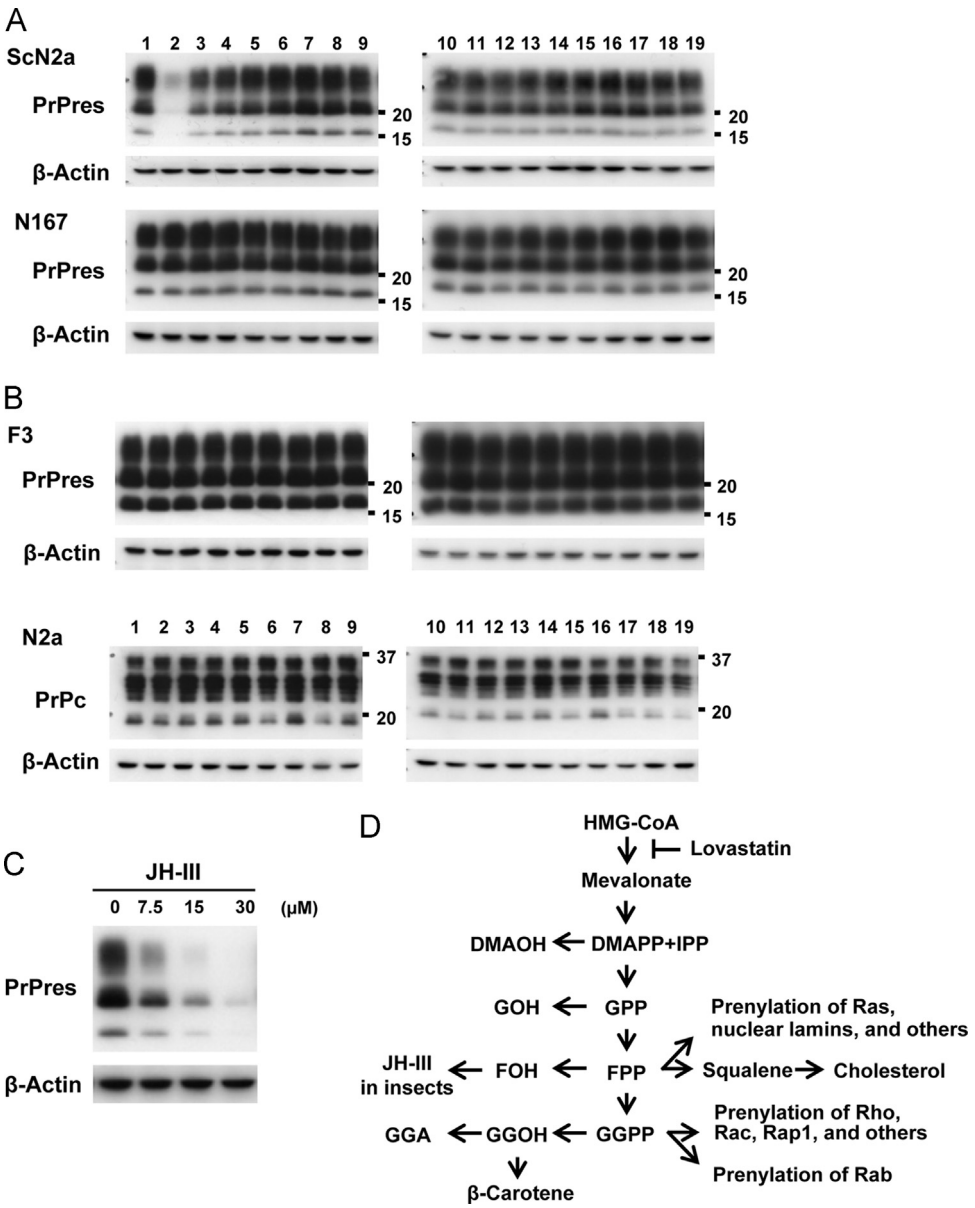

Fig. 1. Antiprion activities of insect-derived substances. (A) Immunoblotting of abnormal protease-resistant prion protein (PrPres) in three distinct prion strain-infected cells (ScN2a, N167, F3) treated with insect-derived substances. Lanes 1 and 10 correspond to the vehicle control. Lanes 2–9 and 11–19 respectively correspond to juvenile hormone III (JH-III), methoprene, ponasterone A, α-ecdysone, 20-hydroxyecdysone, allatotropin, allatostatin A, muristerone A, adipokinetic hormone G, leucokinin I, leucokinin III, allatostatin A, corazonin, drosocin, cecropin A, proctolin, and methoprene acid. Cells were treated for three days with 7.5-μg/mL insect-derived substances. Signals for β-actin are shown as controls for the integrity of samples used for PrPres detection. Molecular size markers on the right denote sizes in kilodalton (kDa). (B) Immunoblotting of normal prion protein (PrPc) in uninfected cells (N2a) treated with insect-derived substances. Cells were treated as described in (A). (C) Immunoblotting of PrPres in ScN2a cells treated with the indicated doses of JH-III. (D) Isoprenoid biosynthesis pathway.