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Anti-fibrillogenic and fibril-destabilizing activity of nicotine in vitro: Implications for the prevention and therapeutics of Lewy body diseases

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Abstract

The aggregation of alpha-synuclein (αS) has been implicated as a critical step in the development of Lewy body diseases (LBD) and multiple system atrophy (MSA). Both retrospective and prospective epidemiological studies have consistently demonstrated an inverse association between cigarette smoking and Parkinson's disease (PD). We used fluorescence spectroscopy with thioflavin S, electron microscopy and atomic force microscopy to examine the effects of nicotine, pyridine, and *N*-methylpyrrolidine on the formation of αS fibrils (fαS) from wild-type αS (αS (WT)) and A53T mutant αS (A53T) and on preformed fαSs. Nicotine dose-dependently inhibited the fαS formation from both αS (WT) and A53T. Moreover, nicotine dose-dependently destabilized preformed fαSs. These effects of nicotine were similar to those of *N*-methylpyrrolidine. The anti-fibrillogenic activity of nicotine may be exerted not only by the inhibition of fαS formation but also by the destabilization of preformed fαS. Additionally, this effect may be attributed to *N*-methylpyrrolidine moieties of nicotine.
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Keywords: Lewy body diseases; Nicotine; α-Synuclein fibrils; Electron microscopy; Atomic force microscopy

Introduction

There are numerous findings suggesting a seminal role for α-synuclein (αS) in a group of neurodegenerative diseases that include Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA) (Paleologou et al., 2005). Firstly, αS is a component of Lewy bodies and neurites in PD and DLB and of glial and neuronal cytoplasmic inclusions in MSA (inclusions present in brain regions that are functionally damaged) (Paleologou et al., 2005). Secondly, three missense αS mutations are linked to rare forms of early-onset familial PD (Kruger et al., 1998; Polymeropoulos et al., 1997; Zarranz et al., 2004). Thirdly, αS forms toxic oligomers or fibrils (Lashuel et al., 2002; Volles and Lansbury, 2003). Fourthly, raised αS expression in cells promotes mitochondrial deficit and cell death, enhances susceptibility to oxidative stress (Ostrerova et al., 1999; Hsu et al., 2000), and can damage lysosomes (Stefanis et al., 2001) and the Golgi apparatus (Gosavi et al., 2002). Finally, increased human αS expression in transgenic flies and mice is accompanied by neuronal dysfunction and loss of synaptic terminals and/or neurons, the formation of lesions similar to those found in PD brain, and the development of motor abnormalities (Feany and Bender, 2000; Kahle et al., 2000). Also, virus-induced over-expression model also revealed the same pathological change of PD (Kirik et al., 2003; Yamada et al., 2004). Taken together, all these studies provide strong evidence for a central role for αS deposition in the pathogenesis of these neuro-degenerative disorders.

Cigarette smoking is consistently associated with a reduced risk of PD, having been reported in prospective studies (Hernan et al., 2001), retrospective case-control studies (Hernan et al., 2002), twin studies (Wirdefeldt et al., 2005), and studies of case-sibling pairs (Scott et al., 2005). In fact, not only cigarette smoke, but also nicotine gum and nicotine patches can decrease the tremors and bradykinesia of PD (Fagerstrom et al., 1994; Ishikawa and Miyatake, 1993). Nicotine in tobacco provokes health problems in humans, but in its pure form it has the potential to be a valuable pharmaceutical agent (Jarvick, 1991). Some neuroprotective effects of nicotine have been already reported. First, nicotine stimulates striatal dopamine neurons

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that are selectively damaged in PD (Grady et al., 1992; Zhou et al., 2001). Second, nicotine exposure protects against neuronal insult in experimental models (Quik and Kulak, 2002; O'Neill et al., 2002; Jeyarasasingam et al., 2002). However, few studies on the direct effects of nicotine for αS in vitro have been reported.

Based on a nucleation-dependent polymerization model to explain the mechanism of β-amyloid fibrils (fAβ) formation in vitro (Jarrett and Lansbury, 1993; Lomakin et al., 1997; Naiki et al., 1997; Naiki and Gejyo, 1999), we previously reported that nicotine inhibits fAβ formation from amyloid β-protein (Aβ) and fAβ extension dose-dependently in vitro (Ono et al., 2002a). Moreover, we reported that nicotine also destabilizes preformed fAβ(1–40) and fAβ(1–42) in a concentration-dependent manner within a few hours at pH 7.5 at 37 °C, based on fluorescence spectroscopic analysis with thioflavin T and electron microscopic studies (Ono et al., 2002a). Our systematic in vitro studies including this study indicated that the overall activity of the antiamyloidogenic molecules may be on the order of nordihydroguaiaretic acid (NDGA) > rifampicin (RIF) = tetracycline > poly(vinylsulfonic acid, sodium salt) = 1,3-propanedisulfonic acid, disodium salt > nicotine (Ono et al., 2002a,b). The time course of αS aggregation also fulfills all the criteria of the nucleation-dependent model, characterized by an initial lag phase reflecting nucleation and a subsequent growth phase culminating in a steady state (Wood et al., 1999). Structurally, the in vitro αS aggregates resemble material derived from disease-affected brains, i.e., they exhibit an amyloid-like fibrillar morphology (Serpell et al., 2000). The mutated forms, especially the A53T mutated form (A53T) of αS were shown to have higher aggregation rates than wild-type form (WT) (El-Agnaf et al., 1998b; Conway et al., 1998). Recently, we showed that antioxidant compounds such as NDGA and RIF dose-dependently inhibited the formation of αS fibrils (fαS) and destabilized preformed fαS as found in fAβ (Ono and Yamada, 2006).

In this study, we used fluorescence spectroscopy with thioflavin S (ThS), electron microscopy (EM) and atomic force microscopy (AFM) to examine the effects of nicotine and two components of nicotine, i.e., pyridine and *N*-methylpyrrolidine (M-pyrro) on the formation and destabilization of preformed fαS at pH 7.5 at 37 °C in vitro.

Materials and methods

Preparation of αS and fαS solutions

Wild-type αS (αS (WT))(lot number 121303AS) and A53T mutant αS (A53T) were purchased from Recombinant Peptide Technologies (LLC, GA, USA). Fresh, non-aggregated fαS was obtained by polymerizing fresh αS just before the destabilization reaction. The reaction mixture contained 140 μM αS, 20 mM Tris buffer, pH 7.5, and 100 mM NaCl. After incubation at 37 °C for 6 days under agitated conditions, the formation reaction proceeded to equilibrium as measured by the fluorescence of ThS. In the following experiments, the concentration of fαS in the final reaction mixture was regarded as 140 μM.

Fluorescence spectroscopy

A fluorescence spectroscopic study was performed using a Hitachi F-2500 fluorescence spectrophotometer as described previously (Ono and Yamada, 2006). Optimum fluorescence measurements of fαS were obtained at the excitation and emission wavelengths of 440 and 521 nm, respectively, with containing the reaction mixture 5 μM ThS (Wako Pure Chemical Industries, Osaka, Japan) and 50 mM of glycine–NaOH buffer, pH 8.5.

Electron microscopy (EM)

Reaction mixtures were spread on carbon-coated grids, negatively stained with 1% phosphotungstic acid, pH 7.0, and examined under a JEM-1210 electron microscope (JEOL Ltd., Tokyo, Japan) with an acceleration voltage at 75 kV.

Atomic force microscopy (AFM)

fαS solutions were mixed gently prior to removal of aliquots for AFM analysis. The aliquots (20 μl) were applied to a freshly cleaved muscovite mica substrate and kept at room temperature for 10 min. The mica surface was then rinsed with Millipore-filtered water (3 × 50 μl) to remove loosely bound protein and dried under a stream of CO2. The sample was imaged immediately using a Nanoscope IIIa controller (Digital Instruments, Santa Barbara, CA) with a Multimode scanning probe microscope equipped with a JV scanner. All measurements were carried out in the tapping mode under ambient conditions using single-beam silicon cantilever probes. The image size of AFM was usually 512 × 512 pixels. At least four regions of the mica surface were examined to confirm the homogeneity of the structures throughout the sample. Section analysis was accompanied by drawing a line over the individual species and measuring the distance from the mica surface to the top of the structure. This was done 10 times and an average was obtained only when the fibril structure was confirmed.

Polymerization assay

Polymerization assay was preformed as described elsewhere (Ono and Yamada, 2006). Briefly, the reaction mixture contained 140 μM αS (WT or A53T); 0, 0.1, 1, 10, 50 or 100 μM NDGA, RIF, nicotine (Sigma Co., St. Louis, MO), pyridine (Nacalai Tesque, Kyoto, Japan) or M-pyrro (Tokyo Kasei Kogyo, Tokyo, Japan); 20 mM Tris buffer, pH 7.5; and 100 mM NaCl.

30 μL aliquots of the mixture were put into oil-free PCR tubes (size; 0.5 mL, Takara Shuzo Co. Ltd., Otsu, Japan). The reaction tubes were put into an incubator (IK100J, Yamato Co. Ltd., Tokyo, Japan). Starting at 4 °C, the plate temperature was elevated at maximal speed, to 37 °C. After they were stirred with micro-beads for 0–6 days as indicated in each figure, the reaction was stopped by placing the tubes on ice. From each reaction tube, triplicate 5-μL aliquots were removed, then subjected to fluorescence spectroscopy and the mean of each

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