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Selegiline-functionalized, PEGylated poly(alkyl cyanoacrylate) nanoparticles: Investigation of interaction with amyloid-β peptide and surface reorganization

Benjamin Le Droumaguet[1], Hayfa Souguir[1], Davide Brambilla, Romain Verpillot, Julien Nicolas*, Myriam Taverna, Patrick Couvreur, Karine Andrieux**

Laboratoire de Physico-Chimie, Pharmacotechnie et Biopharmacie, Univ. Paris-Sud 11, UMR CNRS 8612, Faculté de Pharmacie, 5 rue Jean-Baptiste Clément, 92296 Châtenay-Malabry, France

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder for which the research of new treatments is highly challenging. Since the fibrillogenesis of amyloid-β peptide 1–42 ($A\beta_{1-42}$) peptide is considered as a major cause of neuronal degeneration, specific interest has been focused on aromatic molecules for targeting this peptide. In this paper, the synthesis of selegiline-functionalized and fluorescent poly(alkyl cyanoacrylate) nanoparticles (NPs) and their evaluation for the targeting of the $A\beta_{1-42}$ peptide are reported. The synthetic strategy relied on the design of amphiphilic copolymers by tandem Knoevenagel–Michael addition of cyanoacetate derivatives, followed by their self-assembly in aqueous solutions to give the corresponding NPs. Different cyanoacetates were used: (i) hexadecyl cyanoacetate (HDCA) to form the hydrophobic core of the NPs; (ii) rhodamine B cyanoacetate (RCA) for fluorescent purposes; (iii) methoxypoly(ethylene glycol) cyanoacetate (MePEGCA) for stealth properties and (iv) selegiline-poly(ethylene glycol) cyanoacetate (SelPEGCA) to obtain the desired functionality. Two different amphiphilic copolymers were synthesized, a selegiline-containing copolymer, P(MePEGCA-*co*-SelPEGCA-*co*-HDCA), and a rhodamine-labelled counterpart, P(MePEGCA-*co*-RCA-*co*-HDCA), further blended at variable ratios to tune the amount of selegiline moieties displayed at the surface of the NPs.

Optimal formulations involving the different amphiphilic copolymers were determined by the study of the NP colloidal characteristics. Interestingly, it was shown that the zeta potential value of the selegiline-functionalized nanoparticles dramatically decreased, thus emphasizing a significant modification in the surface charge of the nanoparticles. Capillary electrophoresis has then been used to test the ability of the selegiline-functionalized NPs to interact with the $A\beta_{1-42}$ peptide. In comparison with non functionalized NPs, no increase of the interaction between these functionalized NPs and the monomeric form of the $A\beta_{1-42}$ peptide was observed, thus highlighting the lack of availability of the ligand at the surface of the nanoparticles. A mechanism explaining this result has been proposed and was mainly based on the burial of the hydrophobic selegiline ligand within the nanoparticles core.

1. Introduction

Alzheimer's disease (AD) is a severe neurodegenerative illness affecting more and more aging population over the world. AD represents the most common cause of dementia and is characterized by a progressive, but irreversible deterioration of cognitive functions and a loss of memory (Querfurth and LaFerla, 2010). Although the mechanisms leading to these dysfunctions are still unclear and under debate (Aliev et al., 2004; de la Torre, 2004; Korolainen et al., 2010), the disease is physiologically characterized by two main pathological features. These hallmarks are: (i) the intracellular accumulation of the hyperphosphorylated tau protein in the neurons and (ii) the progressive production and aggregation of β-amyloid peptide (Aβ) (Aguzzi and O'Connor, 2010; Panza et al., 2009), the latter being considered as the main cause of AD (Gralle et al., 2009). Neurons produce Aβ peptides with a variable number of amino-acids and the Aβ peptide 1–42 ($A\beta_{1-42}$) is believed to be the most representative and the most toxic species in AD physiopathology due to its high tendency to spontaneously self-aggregate (Chow et al., 2010; García-Matas et al., 2010).

In the last decades, the pharmaceutical companies have only attempted to combat clinical manifestations of AD. In particular, acetylcholinesterase (AchE) inhibitors (Birks et al., 2009; Munoz-

* Corresponding author. Tel.: +33 1 46 83 58 53; fax: +33 1 46 83 59 46.
** Corresponding author. Tel.: +33 1 46 83 58 12; fax: +33 1 46 83 59 46.
E-mail addresses: julien.nicolas@u-psud.fr (J. Nicolas), karine.andrieux@u-psud.fr, karine.andrieux@cep.u-psud.fr (K. Andrieux).
[1] These authors contributed equally to this work.

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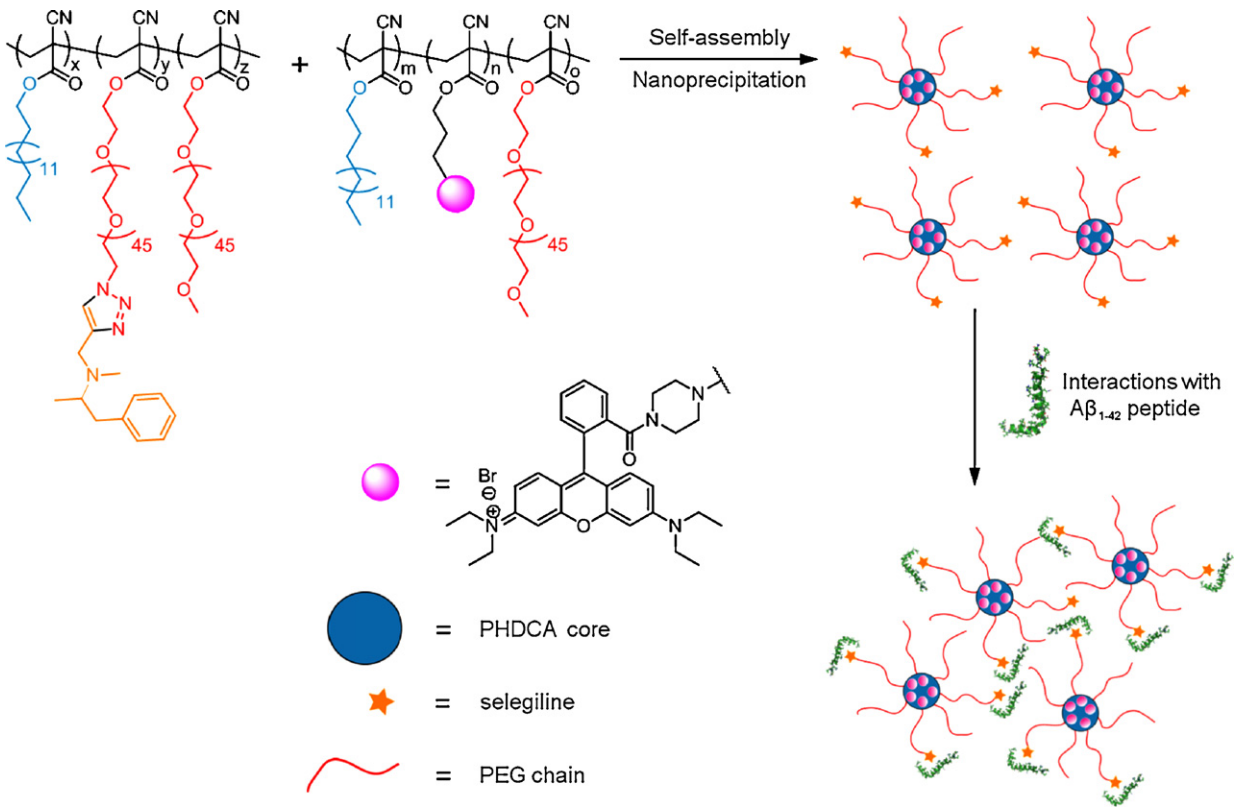

Fig. 1. General approach for the synthesis of selegiline-functionalized, PEGylated poly(alkyl cyanoacrylate) nanoparticles and their possible interaction with the HiLyte Fluor™ 488 labelled amyloid-β 1–42 ($A\beta_{1-42}$) peptide.

Torrero, 2008; Sugimoto et al., 1995) and *N*-methyl-D-aspartate (NMDA) receptor antagonists (Kemp and McKernan, 2002; Parsons et al., 1999; Reisberg et al., 2003) have been widely used but without significant success. Unfortunately, no efficient treatment aiming at the eradication of AD has been proposed so far.

Recently, different studies promoted the utilization of small aromatic molecules for targeting the $A\beta_{1-42}$ peptide, such as curcumine (Garcia-Alloza et al., 2007; Lim et al., 2001; Ono et al., 2004) and its derivatives (Narlawar et al., 2008), Thioflavine T (Xie et al., 2006), Congo red (Carter and Chou, 1998; Lorenzo and Yankner, 1994; Virginia, 2002) and their analogues such as Chrysamine G (Klunk et al., 1998; Lee, 2002; Virginia, 2002) and X34 (Christopher et al., 2001; Styren et al., 2000). These molecules have shown a certain efficiency to hinder, or even to stop, the oligomerization of the $A\beta_{1-42}$ peptide and thus the production of oligomers and/or fibrils, which are commonly considered as the toxic species for neuronal cells. These ligands have also been extensively used as tracers of the presence of senile plaques in the brain due to their fluorescent properties. Moreover, they can be modified with radiolabelled elements for diagnostic purposes (Dezutter et al., 1999a, 1999b; Nordberg, 2004; Wang et al., 2002, 2004). Unfortunately, these compounds do not overpass the blood–brain barrier (BBB). To circumvent this crucial problem, researchers have developed three main strategies: (i) the chemical modification of ligands to make them able to cross the BBB, (ii) their encapsulation into nanoparticles (NPs) and (iii) their ligation to NPs (Narlawar et al., 2008; Sun et al., 2010).

Among the pool of efficient ligands discovered so far, we have focused our attention on selegiline, an aromatic molecule that has been employed to slow down the progression of the Parkinson's disease (Tetrud and Langston, 1989), but that has been more importantly used as monoamine oxidase-B inhibitor for the treatment of AD (Sano et al., 1997; Tom, 2000; Wilcock et al., 2002). Selegiline also exhibited a certain affinity for the $A\beta_{1-42}$ peptide (Re et al., 2010). In this study, we presented the synthesis of selegiline-functionalized and fluorescent poly(alkyl cyanoacrylate) nanoparticles for $A\beta_{1-42}$ peptide targeting and anti-fibrillogenesis purposes. By capturing monomeric peptide at the surface of these nanoparticles, we aim to inhibit its fibrillogenesis. The synthesis strategy relied on the dual modification of poly[methoxypoly(ethylene glycol) cyanoacrylate-*co*-poly(hexadecyl cyanoacrylate)] (P(MePEGCA-*co*-HDCA)) copolymers (Nicolas and Couvreur, 2009; Peracchia et al., 1999) to introduce: (i) selegiline moieties at the extremity of PEG chains and (ii) rhodamine B probes within the copolymer structure. The P(MePEGCA-*co*-HDCA) copolymer scaffold has been selected due to its successful ability to cross the BBB (Calvo et al., 2001; Garcia-Garcia et al., 2005a, 2005b). Moreover, we took advantage of a recent study that demonstrated the efficient derivatization of such copolymers with azido-functional groups allowing subsequent reaction with alkyne-containing ligands through copper-catalyzed azide–alkyne cycloaddition (CuAAC) (Nicolas et al., 2008). Functionalization was undertaken with selegiline by CuAAC *via* its native alkyne group. NPs were obtained from the self-assembly of different ratios of selegiline-functionalized and rhodamine B-tagged copolymers in order to tune the amount of selegiline moieties displayed at their surface (Fig. 1). The resulting functionalized nanoparticles, obtained by the nanoprecipitation technique, were characterized by dynamic light scattering (DLS) and zeta potential (ζ) measurements. Finally, we used capillary electrophoresis (CE) to monitor the interaction of the selegiline-functionalized NPs with the $A\beta_{1-42}$ peptide (Brambilla et al., 2010b).