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Inhibitory effect of human serum albumin on Cu-induced $A\beta_{40}$ aggregation and toxicity

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ABSTRACT

It has been suggested that the aggregation and cytotoxicity of amyloid-β (Aβ) peptide with transition-metal ions in neuronal cells is involved in the development and progression of Alzheimer's disease (AD). As the most abundant protein in blood plasma and in cerebrospinal fluid, human serum albumin (HSA) can bind Aβ in vivo and subsequently inhibit Aβ fibril growth. However, the roles of albumin in Cu-induced Aβ aggregation and toxicity, and its potential biological relevance to AD therapy, were not stressed enough. Here, we showed that HSA was capable of binding Cu (I) with much higher affinity than Aβ, competitively inhibiting the interaction of Aβ and Cu ions. In the presence of biological reducing agent ascorbate, HSA inhibited Cu (II)/Cu (I)-mediated $A\beta_{40}$ aggregation, reactive oxygen species production, and neurotoxicity. However, in the absence of Cu (II)/Cu (I), HSA could not effectively inhibit $A\beta_{40}$ aggregation and neurotoxicity at 24 h (or less) incubation time, but decreased $A\beta_{40}$ aggregation at much longer incubation (120 h). Our data suggested that through competitively decreasing Cu–Aβ interaction, HSA could effectively inhibit Cu (II)/Cu (I)-induced $A\beta_{40}$ aggregation and neurotoxicity, and play important roles in regulating redox balance as well as metal homeostasis in AD prevention and therapy.

1. Introduction

Alzheimer's disease (AD) is one of the most common progressive neurodegenerative disorders in the elderly. Many studies have demonstrated that excess amyloid β-peptides (Aβ) in the brain are believed to be the culprits in the neurodegeneration of AD (Hamley, 2012; Rauk, 2009). The aggregation of Aβ peptides and generation of reactive oxygen species are the two markers of AD and can be responsible for the early oxidative damage observed in AD (Hamley, 2012; Hureau and Dorlet, 2012; Rauk, 2009; Zhu et al., 2007).

The effects of Cu on Aβ aggregation and toxicity have attracted broad attention and are extensively explored (Chen et al., 2011; Hureau and Dorlet, 2012; Sarell et al., 2010). It is suggested that aberrant homeostasis of cerebral Cu (II) may contribute to the AD pathogenesis by facilitating the formation of amyloid deposits and enhancing the generation of reactive oxygen species, resulting in oxidative stress, neurotoxicity and cognitive impairment (Hureau and Dorlet, 2012; Sarell et al., 2010). Moreover, the level of Cu is markedly higher within AD neocortex than within age-matched controls, and are even further concentrated within plaque deposits (Hureau and Dorlet, 2012). It has been found that Aβ spontaneously binds Cu (II) or Cu (I) *via* histidine residues with high affinity (Hureau and Dorlet, 2012; Nakamura et al., 2007). In the presence of the reduced transition metal ions (Cu (I)) and endogenous reducing agent (vitamin C), there is spontaneous generation of neurotoxic partially reduced oxygen species, for example, H_2O_2, hydroxyl radical (•OH) (Nakamura et al., 2007; Pramanik et al., 2011).

Human serum albumin (HSA) is the most abundant protein in blood plasma and in cerebrospinal fluid (CSF) with typical concentrations of ~0.6 mM and ~4 μM, respectively (Milojevic et al., 2009; Rózga et al., 2007a, 2007b; Stanyon and Viles, 2012). HSA constitutes 35–80% of the total proteins in CSF. HSA has been proposed to be involved in the transport of the essential metal Cu which binds strongly to HSA (Rózga et al., 2007b). The presence of Cu, Aβ and HSA in the CSF suggests that a Cu-transfer reaction should be physiologically relevant. Recent study showed that physiological and micromolar levels of HSA inhibited the kinetics of Aβ fibrillization, significantly increasing the lag time and decreasing the total amount of fibrils produced (Milojevic et al., 2009; Stanyon and Viles, 2012). Thus, HSA was a potent inhibitor of Aβ self-association and this novel function of HSA was of

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potential therapeutic interest for the treatment of AD. However, the roles of albumin in Cu-induced Aβ aggregation and toxicity, and its potential biological relevance to AD, were little known.

In the present work, we assessed the relative Cu (I)-binding affinity between the two molecules (Aβ and HSA), as well as the effect of HSA on Cu-mediated $A\beta_{40}$ aggregation, reactive oxygen species production and toxicity on neuroblastoma cells. The results indicated that endogenous HSA was able to alleviate the cytotoxicity induced by Aβ and Cu. This information may be a foundation to develop a potential strategy to treat AD.

2. Materials and methods

2.1. Materials

Human serum albumin (HSA), ascorbate, bicinchoninic acid (BCA) and thioflavin T (ThT) were purchased from Sigma. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Gibco BRL (Gaithersburg, MD, USA). Soluble $A\beta_{1-40}$ peptides ($A\beta_{40}$) were synthesized by ChinaPeptides (Shanghai, China) with >95% purity. All solvents and other reagents were the highest purity commercially available.

2.2. Competitive binding Cu (I) by HSA and Aβ

As a colorimetric Cu (I) chelator, BCA combines with Cu (I) to form a stable Cu (I)–BCA complex, which exhibits absorption maxima at 562 nm. Therefore, the transfer of Cu (I) from BCA to the protein can be monitored by the decrease in characteristic absorbance of Cu (I)–BCA complex at 562 (Du et al., 2014). In the presence of reducing agent (ascorbate), Cu (I) is reduced from Cu (II)($CuSO_4$). Ascorbate concentration was kept at 0.3 mM to maintain the oxidation state of Cu (I) in the reaction mixture. After the addition of 1 mol equivalent of HSA or $A\beta_{40}$ into the mixture with a final concentration of 20 μM Cu (I) and 60 μM BCA in PBS (pH 7.0), absorbance of Cu (I)–BCA at 562 nm was recorded. To ensure the equilibrium was reached, the mixture was incubated at room temperature for at least 1 h (no additional change in absorption was observed).

2.3. ThT fluorescence detection of Aβ aggregates

ThT associates rapidly with aggregated Aβ peptides, giving rise to an excitation maximum at 440 nm and enhanced emission at 483 nm (Du et al., 2014; Lu et al., 2015; Stanyon and Viles, 2012). In the presence or absence of Cu (II), Aβ (20 μM) was coincubated with HSA (20 μM) in 20 mM PBS (pH 7.0) for relative short (8, 16 and 24 h) and long (120 h) time. Aliquots of Aβ samples were removed and mixed with ThT in PBS and the fluorescence was measured with a fluorescence spectrophotometer.

2.4. Cell viability assay

To assess the effects of HSA on Cu–$A\beta_{40}$ induced toxicity, human neuroblastoma SH-SY5Y cells were grown at 37 °C in PBS containing DMEM (without foetal calf serum) and then exposed for 24 h to H_2O_2, or freshly prepared Cu (II)–$A\beta_{40}$ (20 μM) in the presence of different concentrations of HSA (0, 20 or 50 μM) with ascorbate (0.3 mM). After that, cell viability was measured by using MTT assay (Chen et al., 2009; Luo et al., 2013; Meloni et al., 2008).

2.5. Reactive oxygen species measurement

In the presence or absence of Cu (II), Aβ (20 μM) was coincubated with HSA (20 μM) in 20 mM PBS (pH 7.0) for 24 h. The reaction was started by the addition of 0.3 mM ascorbate. Hydroxyl radical (•OH) production was monitored by fluorescence spectroscopy (λ_{ex}= 390 nm, λ_{em}=460 nm) using the •OH scavenging compound coumarin as described previously (Luo et al., 2013). Xylenol orange assay was performed for the measurement of H_2O_2 formation, with the characteristic absorbance at 560 nm (Pramanik et al., 2011).

2.6. Statistical analysis

All of the experiments were performed at least three times. The results were reported as the means ± S.D. of at least triplicate determinations. One-way ANOVA was used for statistical analyses, and $P < 0.05$ was considered significant.

3. Results

CSF contains micromolar levels of HSA but nanomolar levels of Aβ. HSA binds Cu (II) with pM affinity using its amino terminal Cu (II)-binding motif, which is much stronger than the affinity reported between Cu (II) and Aβ (Rózga et al., 2007a, 2007b). Unlike in plasma where extracellular copper is presumed to be Cu (II), the concentration of ascorbate present in CSF and extracellular fluid is sufficient to generate Cu (I) (Du et al., 2014; Guilloreau et al., 2007; Meloni et al., 2008; Perrone et al., 2010). Therefore, Aβ preferentially bound Cu (I) rather than Cu (II) in vivo, and ascorbate-Cu (II) system was throughout used in this work and Cu (II)/Cu (I) was present.

3.1. Competitive binding Cu (I) by HSA and Aβ

The capacity of $A\beta_{40}$ to compete for Cu (I) with BCA was compared. Previously, Cu (I) was suggested to be ligated by the His13 and His14 in Aβ (Hureau and Dorlet, 2012; Nakamura et al., 2007). As shown in Fig. 1A, $A\beta_{40}$ was unable to remove Cu (I) from the Cu (I)–BCA complex, while HSA could remove Cu (I) from both Cu (I)–BCA and Aβ–Cu (I)–BCA complexes. Therefore, HSA bound Cu (I) more tightly than $A\beta_{40}$, which was similar to the higher binding property of selenoprotein P than $A\beta_{42}$ (Du et al., 2014). The much higher binding affinity of Cu toward HSA compared with $A\beta_{40}$ indicated that HSA might modulate the Cu-induced aggregation and neurotoxicity of $A\beta_{40}$.

3.2. HSA attenuated Cu-induced Aβ aggregation

The aggregation degrees of $A\beta_{40}$ incubated with different additives (Cu and HSA) were examined by ThT fluorescence assay. As shown in Fig. 1B, in the presence of biological reducing agent ascorbate, Cu (II)/Cu (I)-induced Aβ aggregation was more significant than Aβ self-aggregation, in good agreement with the previous observation that copper ions promoted the fibrillization of Aβ (Hureau and Dorlet, 2012; Nakamura et al., 2007). However, HSA could effectively inhibit Cu-mediated $A\beta_{40}$ aggregation at all of the selected time incubation, while HSA had less effect on $A\beta_{40}$ self-aggregation at short (8, 16 and 24 h) time incubation and inhibited aggregation only at longer (120 h, or more) time incubation (Fig. 1C). Moreover, HSA could significantly inhibit Cu-mediated $A\beta_{40}$ aggregation in a dose-dependent manner (Fig. S1A).

The different effects of HSA on Cu-mediated Aβ aggregation and Aβ self-aggregation (absence of Cu) was attributed to different mechanisms. In Aβ self-aggregation, HSA binds to the monomeric Aβ effectively, traps them in a nonfibrillar form and so that they are not available to form fibers. The lag phase in fibril growth is strongly affected by the Aβ concentration, which would explain the reduction in lag times observed (Milojevic et al., 2009; Stanyon and Viles, 2012). On the other hand, HSA is also responsible for the