Age-associated changes in amyloid-β and formaldehyde concentrations in cerebrospinal fluid of rhesus monkeys

DEAR EDITOR,

Rhesus monkeys (*Macaca mulatta*) are valuable experimental animals for studies on neurodegenerative diseases due to their evolutionarily close relationship to humans (Zhang et al., 2014). Rhesus monkeys also display similar hallmarks of aging and neurodegeneration as humans, including formation of senile plaques in the brain (Beckman et al., 2019; Paspalas et al., 2018). However, changes in formaldehyde (FA) levels in the cerebrospinal fluid (CSF) of rhesus monkeys with aging have not been reported. Additionally, whether changes in CSF FA are correlated with changes in amyloid-β (Aβ) concentrations have not yet been explored. Here, the CSF levels of $A\beta_{40}$, $A\beta_{42}$, and FA were measured in 56 rhesus monkeys of different ages, ranging from 4 to 26 years old. Results revealed significant declines in $A\beta_{40}$ and $A\beta_{42}$, and an increase in FA with age. Interestingly, the increase in FA levels was negatively correlated with $A\beta_{40}$ and $A\beta_{42}$ concentrations in aged rhesus monkeys but not in young and middle-aged monkeys. These results appear to parallel changes seen within human aging, i.e., decreased levels of CSF Aβ and increased levels of FA in normal aged adults and Alzheimer's disease (AD) patients. These findings further indicate that rhesus monkeys are a reliable model for studying age-related neurological disorders such as AD and suggest that FA is an important factor in AD development and may be used as a diagnostic indicator of such disease.

Aβ is a secreted peptide of unknown physiological function that is produced by sequential cleavage of β-amyloid precursor protein (APP) by β-secretase and γ-secretase (Vassar, 2005). Most Aβ is produced in the brain, but it also effluxes into the CSF and plasma, appearing in relatively high and low concentrations, respectively. Aβ occurs in multiple forms ranging from 38 to 43 amino acids in length (Perrin et al., 2009). Among these, $A\beta_{40}$ is the most abundant species, but $A\beta_{42}$ is essential for initiating Aβ aggregation and is considered central to the amyloid cascade hypothesis of AD (Hardy and Selkoe, 2002). Soluble Aβ (including monomers and a few oligomers and protofibrils) in CSF can be used as a diagnostic indicator for certain neurological diseases. For example, AD patients exhibit a significant decrease in soluble Aβ in their CSF, indicating a portion of soluble Aβ is deposited in brain tissue to form senile plaques (Fagan et al., 2006; Irie, 2020; Lana et al., 2019; Mattsson et al., 2009; Shaw et al., 2009).

There is compelling evidence that suggests FA is related to AD pathology, both *in vivo* and *in vitro*. Several studies have found that FA concentration in the human body increases with age, and concentrations of FA in urine, blood, CSF, and brain tissue of AD patients are significantly higher than those in the control group at the same age (He et al., 2010; Tong et al., 2013). In addition, FA concentration in the urine of AD patients is negatively correlated with cognitive level (Tong et al., 2017; Tong et al., 2011). Therefore, FA is considered to be closely related to the occurrence and development of AD (Tulpule and Dringen, 2013; Wang et al., 2019). In rodent studies, elevated FA can lead to memory impairment, Tau protein hyperphosphorylation, and neuronal loss; rodent animal models of AD also show an imbalance in FA metabolism and

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elevated FA *in vivo* (Qiang et al., 2014; Tong et al., 2013; Yang et al., 2014a). In non-human primate (NHP) studies, elevated FA levels not only lead to impaired memory, but also to the occurrence of all the pathological hallmarks of human AD in the brain, including senile plaques, neurofibrillary tangles, neuronal loss, and glial proliferation (Yang et al., 2014b; Zhai et al., 2018). Studies have shown that very low concentrations of FA can promote the aggregation of Aβ and the formation of structures similar to senile plaques *in vitro* (Chen et al., 2006; Rizak et al., 2014). Co-incubation of FA and Tau protein can increase the diameter of Tau protein particles, and the increase in particle size is positively correlated with FA concentration and incubation time extension (Nie et al., 2005). FA can also induce the formation of hyperphosphorylated Tau protein and neurofibrillary tangles *in vitro* (He et al., 2017).

Animal models play a major role in defining critical disease-related mechanisms and exploring potential therapeutic approaches in neurodegenerative diseases such as AD (Heuer et al., 2012). Due to their evolutionarily close relationship to humans, NHPs are essential for the study of age-associated changes in the brain and other central nerve system diseases (Chu et al., 2014; Feng et al., 2019; Qin et al., 2013; Zhang et al., 2014). Studies have indicated that CSF levels of Aβ$_{40}$ and Aβ$_{42}$ decrease significantly with age in cynomolgus and vervet monkeys (Chen et al., 2018; Yue et al., 2014). However, no study has investigated the relationship of FA concentrations in CSF samples from different aged rhesus monkeys or the correlation between FA and Aβ levels in CSF samples. In this study, FA and Aβ levels in CSF samples were measured in different aged rhesus monkeys, and the correlations between FA and Aβ in different age groups were investigated.

Fifty-six rhesus monkeys (*Macaca mulatta*) were selected for CSF collection based on restrictive conditions (age: 4–26 years old; healthy and without any prior experimental operations). The monkeys were divided into three groups depending on age: i.e., young (4–7 years old, *n*=15), middle-aged (10–15 years old, *n*=22), and aged (19–26 years old, *n*=19) group (Supplementary Materials and Methods). For CSF collection, the monkeys were anesthetized with 10 mg/kg body weight of ketamine by intramuscular injection. A 22-gauge spinal needle was inserted into the lumbar interspace at the same level as the palpated iliac crest, and approximately 1.5 mL of CSF (divided into three 0.5 mL fractions) was withdrawn through a lumbar puncture (Supplementary Materials and Methods). The CSF samples were then immediately frozen in liquid nitrogen and stored in a −80°C freezer until analysis.

The levels of Aβ$_{40}$ and Aβ$_{42}$ in CSF were measured using commercial ELISA kits (Aβ$_{42}$ and Aβ$_{40}$ Assay Kits, Cat. No. KHB3441 and KHB3481, respectively, Life Technologies, USA) according to the manufacturer's instructions. Each sample was tested in duplicate. For measurement of FA levels, after centrifugation (20 000 *g*, 4 °C, 15 min), the resulting supernatant fractions were used for analysis of FA by high-performance liquid chromatography with fluorescence

detection (Fluo-HPLC), as described in previous study (Supplementary Materials and Methods) (Tong et al., 2011). For statistical analysis, intergroup differences were evaluated by one-factor analysis of variance followed by Least Square Difference (LSD) tests. The relationships between FA and Aβ concentrations were subsequently analyzed by linear regression. A value of *P*<0.05 was considered significant in all analyses. All statistical analyses were conducted using GraphPad Prism 8 (San Diego, USA).

The concentrations of Aβ$_{40}$ (*P*=0.011, Figure 1A) and Aβ$_{42}$ (*P*=0.044, Figure 1B) in the CSF of aged monkeys were markedly decreased compared to that in middle-aged subjects. These results are in agreement with prior studies, which report a significant decrease in soluble Aβ in the CSF of AD patients and aged monkeys, indicating a portion of soluble Aβ is deposited in brain tissue to form senile plaques (Fagan et al., 2006; Yue et al., 2014). APP, which can be cleaved into Aβ by β-secretase and γ-secretase, is completely homologous between humans and rhesus monkeys (Podlisny et al., 1991). β-amyloid cleaving enzyme-1 (β-secretase, or BACE-1) activity increases significantly with age in mouse, monkey, and human brains (Fukumoto et al., 2004). This causes the production of Aβ to increase with age. However, during the onset of AD in old age, Aβ is deposited in the brain to form senile plaques, and CSF Aβ levels are significantly reduced (Fagan et al., 2006). Soluble Aβ in the CSF of young monkeys should account for all Aβ, as there should be no Aβ deposition in the brain tissue (Kimura et al., 2003). Furthermore, the production of Aβ should also increase with the increase in age. However, after brain tissue deposition or receptor interaction increases (Lustbader et al., 2004), soluble Aβ in CSF decreases instead. That is probably why significant age-associated declines in CSF Aβ$_{40}$ (Figure 1A) and Aβ$_{42}$ (Figure 1B) were found between the middle-aged and aged monkeys, but not between the young monkeys and other groups.

The concentrations of FA in CSF samples of aged monkeys showed a marked elevation compared with that in young (*P*=0.001) and middle-aged (*P*<0.001) monkeys (Figure 1C). The significant increase in FA concentration in the CSF of monkeys with age is consistent with results from human studies (Tong et al., 2015). There are multiple factors that contribute to the endogenous accumulation of FA, including environmental pollution (Clejan and Cederbaum, 1993; Takeuchi et al., 2007), FA-generating enzyme disorders (del Mar Hernandez et al., 2005; Ferrer et al., 2002), and FA-degrading enzyme deficiencies (Ohta and Ohsawa, 2006; Wang et al., 2008). Rhesus monkeys and humans show the same FA metabolism pathway; thus, aging in rhesus monkeys and humans may produce abnormal FA metabolism, leading to an increase in FA in the body, and possibly to an increase in pathology (Liesivuori and Savolainen, 1991; Tulpule and Dringen, 2013; Zhai et al., 2016). Furthermore, a strong causative connection between FA and AD-like pathology and cognitive impairment has been proposed based on our previous studies. Elevated FA not only causes the aggregation of Aβ peptides, Tau hyperphosphorylation, and Tau protein
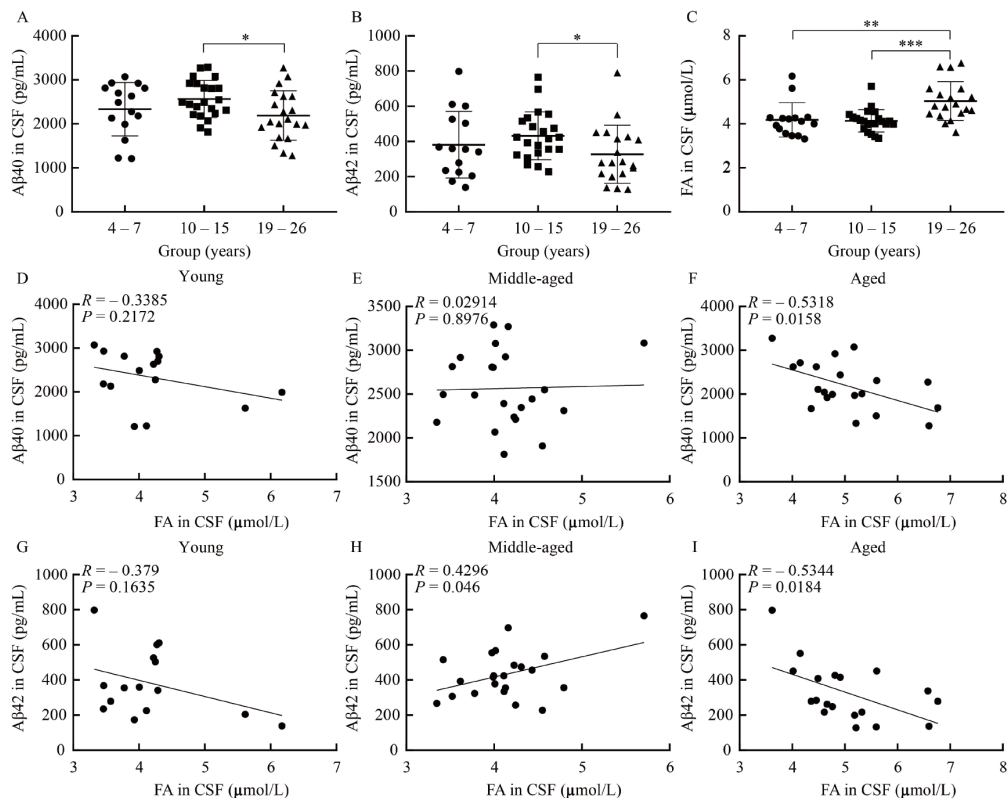
Figure 1 Intergroup analyses of Aβ$_{40}$, Aβ$_{42}$, and FA concentrations in CSF, and correlations between CSF Aβ and FA concentrations in young, middle-aged, and aged rhesus monkeys

A: Intergroup analyses of Aβ$_{40}$ concentrations. B: Intergroup analyses of Aβ$_{42}$ concentrations. C: Intergroup analyses of FA concentrations. D: Correlation between CSF Aβ$_{40}$ and FA concentrations in young group (R=–0.3385, P=0.2172). E: Correlation between CSF Aβ$_{40}$ and FA concentrations in middle-aged group (R=0.02914, P=0.8976). F: Correlation between CSF Aβ$_{40}$ and FA concentrations in aged group (R=–0.5318, P=0.0158). G: Correlation between CSF Aβ$_{42}$ and FA concentrations in young group (R=–0.379, P=0.1635). H: Correlation between CSF Aβ$_{42}$ and FA concentrations in middle-aged group (R=0.4296, P=0.046). I: Correlation between CSF Aβ$_{42}$ and FA concentrations in aged group (R=–0.5344, P=0.0184). Error bars indicate mean± standard deviation (*SD*). *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$. Aβ: β-amyloid; CSF: Cerebrospinal fluid.

polymerization *in vitro* (Lu et al., 2013; Rizak et al., 2014), but also causes pathological and cognitive impairment similar to AD in laboratory animals (Yang et al., 2014a, 2014b; Zhai et al., 2018).

To determine the relationship between Aβ and FA levels in CSF, we explored the correlations among CSF Aβ$_{40}$ and Aβ$_{42}$ concentrations with FA levels (Figure 1D–I). At a nominal significance threshold (P=0.05), Aβ$_{40}$ was correlated with FA concentration in the aged group (P=0.0158, Figure 1F), and Aβ$_{42}$ was correlated with FA concentration in the middle-aged (P=0.046, Figure 1H) and aged groups (P=0.0184, Figure 1I). Each regression coefficient was negative in the aged group (Figure 1F, I); that is, higher concentrations of Aβ$_{40}$ and Aβ$_{42}$ were associated with lower FA levels. However, each regression coefficient was positive in the middle-aged group (Figure 1H); that is, higher concentrations of Aβ$_{42}$ were associated with higher FA levels. Aβ-binding alcohol dehydrogenase (ABAD) is the main alcohol dehydrogenase in mitochondria and is also one of the metabolic enzymes of FA. Combining Aβ with ABAD will inhibit ABAD activity, resulting in mitochondrial dysfunction, which may be one of the reasons for the decrease in FA removal rate in AD patients and in middle-aged monkeys here (Lustbader et al., 2004; Yao et al., 2011). Therefore, to some extent, the increase in Aβ led to FA elevation (Figure 1H). Thus, the significant negative correlation between FA increase and Aβ decrease in the CSF of the aged group only, the time when AD typically develops, indicates that the increase in FA in the brain may also be related to the onset of AD. Such correlations were not observed in the young or middle-aged groups, again suggesting that the increase in endogenic FA is likely related to the development of AD.

In conclusion, for the first time, we described an increase in FA in the CSF of rhesus monkeys with aging and a negative correlation between FA and Aβ concentrations in aged rhesus monkeys. These results not only indicate that rhesus monkeys are good model animals for studying AD but also suggest that FA is an important factor in AD development and may be a diagnostic indicator of such disease.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Z.H.L., X.P.H., X.T.H., and R.Q.H. designed the study. Z.H.L. and X.P.H. performed the experiment. Z.H.L., H.L., and X.T.H. wrote the manuscript with other authors' input. All authors read and approved the final version of the manuscript.

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