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Research Report

Activated protein C inhibits amyloid β production via promoting expression of ADAM-10

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ABSTRACT

Inhibition of Aβ production and clearance of senile plaques have been considered as potential strategies in the treatment of Alzheimer's disease (AD). Activated protein C (APC) is an important factor in the anticoagulant system. However, whether APC can influence the condition of a chronic neurodegenerative process, such as that present in AD, is unknown. In this study, we found that administration of APC on AD Tg2576 mice significantly reduced amyloid β production and helped to facilitate cognitive improvement. APC could also reduce levels of Aβ40 and Aβ42 produced in APPswe cells, an AD cell model. Further results demonstrated that APC did not change the levels of Aβ-degrading enzymes, insulin-degrading enzyme (IDE), or neprilysin (NEP). Next, we found that APC promoted sAPPα and CTFα release and inhibited sAPPβ and CTFβ release, thereby indicating that APC could regulate Aβ secretion by shifting APP processing from the amyloidogenic pathway toward the nonamyloidogenic pathway. Correspondingly, further study revealed that ADAM-10 expression was increased by APC, suggesting that APC inhibits Aβ secretion through stimulating activity of α-secretase. These findings support the idea that APC could hold therapeutic potential in the treatment of AD.

1. Introduction

Alzheimer's disease (AD) is a slowly progressing disease of the brain. It is neuropathologically characterized by the presence of intracellular neurofibrillary tangles (NFTs) caused by abnormally phosphorylated tau protein and extracellular senile plaques containing β-amyloid (Aβ) (Roberson and Mucke, 2006). Aβ is derived from the amyloid precursor protein (APP) through sequential proteolysis. APP is a type I transmembrane protein with a large extracellular domain. It is cut by both the non-amyloidogenic pathway and the amyloidogenic pathway (Buoso et al., 2010). In the non-amyloidogenic pathway, APP is cut by α-secretase within the Aβ sequence, thereby preventing the formation of Aβ (Esch et al., 1990). This step produces a secreted form of APP (sAPPα) and a C-terminal fragment (C83 or α-CTF). C83 is then cleaved by the γ-secretase complex inside the membrane in order to generate p3 and an APP intracellular C-terminal domain (AICD). In the amyloidogenic pathway, APP is cut by β-secretase cleaves at the N-terminal side of Asp1 of the Aβ sequence, thereby producing an N-terminally truncated form of APP (sAPPβ) and a C-terminal membrane-associated fragment (C99 or β-CTF). C99 is then cleaved by the γ-secretase

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complex inside the membrane which generates the Aβ and APP intracellular C-terminal domain (AICD). The tumor necrosis factor α-converting enzyme (TACE/ADAM17) and ADAM-10 have been proposed as an α-secretase (Asai et al., 2003). BACE1 has been shown to be a β-secretase (Vassar et al.,1999). Promoting α-secretase activity (Vingtdeux and Marambaud, 2012) and inhibiting both β-secretase (Ghosh et al., 2008) and γ-secretase activity (Sheng et al., 2009) have been considered an important therapeutic strategy for AD treatment. Moreover, Aβ can be degraded by several peptidases. Insulin-degrading enzyme (IDE) and neprilysin (NEP) are the two principal zinc metallo-endopeptidases that have been identified as being responsible for degradation of Aβ (Wang et al., 2006).

Activated protein C (APC) is an important factor in the anticoagulant system (Mosnier et al., 2007). APC is a serine protease generated from protein C (PC), an inactive plasma zymogen, via proteolysis by a thrombin–thrombomodulin complex on the vascular endothelial cell surface. For many years, the APC pathway has been studied for its role in the clotting process. Other functions of the PC system have been recently investigated. APC has displayed direct cytoprotective activity through mediating cytoprotective alterations in gene expression (Riewald et al., 2002) and controlling activation of several transcription factors that regulate different anti-apoptotic and anti-inflammatory pathways (Joyce et al., 2001). APC has been demonstrated to protect neurons (Guo et al., 2004) and endothelial cells (Finigan et al., 2005) from various types of injury. Importantly, in the CNS system, APC has displayed its ability to limit brain damage in rodent models of ischemia (Zlokovic, 2005), multiple sclerosis (Han et al., 2008), and amyotrophic lateral sclerosis (Zhong et al., 2009) through different mechanisms. However, whether APC can influence a chronic neurodegenerative process like that present in AD is unknown. In this study, we investigated the effects of APC on Aβ production both in vivo and in vitro.

2. Results

2.1. *Administration of APC attenuated Aβ production in Tg2576 transgenic mice*

To investigate the preventative effects of APC against Aβ depositions and memory impairment in the AD model mice, we treated 15-month old Tg2576 transgenic mice with APC for 1 month. Histological observation in APP Tg2576 mice indicated that APC-treated mice had fewer plaques compared to vehicle-Tg mice, as visualized by Thioflavin-S staining (fibrillar Aβ burden), shown in Fig. 1A. As shown in Fig. 1B, APC treatment led to a statistically significant decrease in the levels of total (FA extracted) Aβ40 and Aβ42. The levels of soluble (DEA extracted) Aβ40 and Aβ42 fractions were reduced by 56% (two-tailed t test, $p<0.001$) and 54% ($p<0.001$), respectively, in APC-treated mice, which is notably a significant reduction.

2.2. *Effects of APC in regards to improvement of memory impairment in Tg2576 mice*

We then compared memory deficiency between the treated and non-treated mice using the water maze test. Importantly, treatment with APC for 1 month significantly ameliorated memory dysfunction in the AD model mice. Statistical analysis of data from day 5 showed that APC treatment brought about significant memory-improving effects. Escape latency of the treated group was shorter than that of the non-treated group (Fig. 2A). Escape distance was also reduced by treatment with APC (Fig. 2B). However, there was no significant difference in average speed between the non-treated and the APC-treated groups (data not shown).

2.3. *APC reduced Aβ production in in vitro cell model*

In order to examine the effects of APC on Aβ production, Aβ40 and Aβ42 levels produced in APPswe cells were investigated after APC administration at various concentrations for 48 h. The ELISA method was used to measure both the culture medium supernatant and cell lysate of the cells. The sum of Aβ in culture medium supernatant and cell lysate was calculated as the total Aβ level. As shown in Fig. 3A and B, our results display that APC could significantly decrease levels of Aβ both in the cell lysate and cell culture media of APPswe cells in a dose-dependent manner.

2.4. *APC didn't change the levels of Aβ-degrading enzymes insulin-degrading enzyme (IDE) and neprilysin (NEP)*

Impaired clearance might play an important role in Aβ accumulation in the pathogenesis of AD. Aβ-degrading enzymes, especially insulin-degrading enzyme (IDE) and neprilysin (NEP), have attracted increasingly more attention. Thus, we investigated the effects of APC in the expression levels of both IDE and NEP. Results show that there were no significant changes in IDE and NEP mRNA (Fig. 4A) or protein expression (Fig. 4B) after APC administration, which thereby indicates that there is no evidence of enzymatic Aβ degradation.

2.5. *APC treatment regulates APP processing*

We next investigated Aβ production pathways. First, we assessed full-length APP (APPfl) using Western blot analysis. However, it was shown that there were no significant changes in protein levels of APPfl after APC treatment at different concentrations in APPswe cells, indicating that the effects of APC treatment in reducing Aβ production were not due to the downregulation of APPfl expression levels (Fig. 5). In the amyloidogenic APP processing pathway, APP is sequentially cleaved by β- and γ-secretases to generate Aβ. Conversely, in the non-amyloidogenic APP processing pathway, APP is sequentially cleaved by α- and γ-secretases, which does not generate Aβ. The levels of APP proteolytic products (sAPPα, sAPPβ, CTFα, and CTFβ) were measured to determine whether APC reduced Aβ production by modulating APP processing. Western blot analysis results indicate that APC treatment increased sAPPα and CTFα levels (Fig. 6A) and decreased sAPPβ and CTFβ expression (Fig. 7A) in a dose-dependent manner in APPswe cells. In vivo experiments also demonstrate that administration of APC in Tg2576 mice leads to upregulation of sAPPα and CTFα (Fig. 6B) and downregulation of sAPPβ and CTFβ (Fig. 7B).