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Short Communication

Neuroprotective effects of caffeine against beta-amyliod neurotoxicity: The involvement of glycogen synthase kinase-3β protein

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Abstract

Introduction: The reduction of glycogen synthase kinase-3β protein level may correlate to the neuroprotective effects of antioxidant agents like caffeine. Therefore, we aimed to evaluate the impact of GSK-3β protein on neuroprotective effects of caffeine in the SHSY5Y cells exposed to beta-amyloid.

Methods: We incubated SHSY5Ycells with beta-amyloid 25–35 and caffeine (0.6 and 1mM) for 24h. Cell viability was determined using MTT test. We used the western blotting technique to measure the glycogen synthase kinase-3β and phosphorylated glycogen synthase kinase-3β protein levels.

Results: Caffeine (0.6 and 1mM) diminished beta-amyloid neurotoxicity and attenuated the beta-amyloid effects on the glycogen synthase kinase-3β protein level in a neuronal culture.

Conclusion: Caffeine neuroprotective effects against beta-amyloid may correlate to glycogen synthase kinase-3β protein.

Keywords:
Caffeine;
Amyloid-beta peptide;
Glycogen synthase kinase;
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Introduction

Caffeine is the most prevalent psychoactive agent used around the world (Panza et al., 2015). Several reports have shown that caffeine exerted a neuroprotective effect in the older ages (Panza et al., 2015; Keshavarz et al., 2017). Animals and human studies have shown that caffeine and caffeine-containing products exerted neuroprotective effects and had beneficial impacts on Alzheimer's disease (AD) (Kolahdouzan and Hamadeh, 2017). Beta-amyloid (Aβ)-induced neurotoxicity is an important hallmark of AD (Decker et al., 2010). Glycogen synthase kinase-3 (GSK-3) is a kinase that mediates the neurotoxic effects of Aβ (Llorens-Marítin et al., 2014) and implicated in the pathophysiology of AD (Llorens-Marítin et al., 2014).

The exact mechanism of caffeine-induced neuroprotection is elusive. Caffeine has profound antioxidant effects in animal models and human studies (Kolahdouzan and Hamadeh, 2017). Oxidative stress activates GSK-3 and may contribute to the neurotoxic effects of Aβ (Kamat et al., 2016).

The manipulation of GSK-3 β signaling may correlate to the neuroprotective effects of antioxidant agents like caffeine. Therefore, we aimed to evaluate the impact of GSK-3β protein on neuroprotective effects of caffeine in the SHSY5Y cells exposed to Aβ.

Materials and methods

We maintained human SHSY5Y neuroblastoma cell line in a medium containing Dulbecco's modified Eagle's medium (DMEM) and Ham's nutrient mixture F-12, 10% fetal bovine serum, 100U/ml penicillin and 100µg/ml streptomycin. In this study, neurotoxicity induced with an aggregated form of Aβ25–35 (20µM). In addition, caffeine at the concentrations of 0.6 and 1mM was used as the neuroprotective agent. We incubated the SHSY5Ycells with Aβ25–35 (20µM) and caffeine (0.6 and 1 mM) for 24h. The study groups (n=4) were as follow: (1) control, (2) Aβ (20µM), (3) Aβ (20µM)+ caffeine (0.6 mM), (4) Aβ (20µM)+ caffeine (1mM), (5) caffeine (0.6mM) and (6) caffeine (1mM).

Cell viability was determined using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) reagent and a microplate reader (Synergy HT, Biotek®, Winooski, VT, USA). After the extraction of total protein, we measured the GSK-3β and phosphorylated (p)-GSK-3β proteins were measured by western blotting.

Statistical analysis

We analyzed the results by one-way analysis of variance (ANOVA) followed by the LSD test using the SPSS software (version 23).

Results

The present study showed that Aβ decreased neuronal viability compared to the control-treated group (P<0.001, Fig.1). However, caffeine (0.6 and 1 mM) reduced the Aβ neurotoxic effects (P<0.001, Fig. 1). Caffeine without Aβ exerted no significant effect on neuronal cell survival (P>0.05, Fig. 1).

Aβ also increased the total GSK-3β levels (P<0.001) and decreased p-GSK-3β levels (P=0.05) compared to the control-treated group (Fig. 2). In contrast, caffeine (0.6 and 1 mM) diminished the Aβ effects on the GSK-3β level in neuronal culture (P<0.001, Fig. 2). Caffeine (0.6 and 1 mM) reduced the p-GSK-3β levels compared to the Aβ-treated group (P<0.001 and P=0.037, respectively; Fig. 2). Caffeine (0.6mM) without Aβ increased GSK-3β level (P<0.001) and

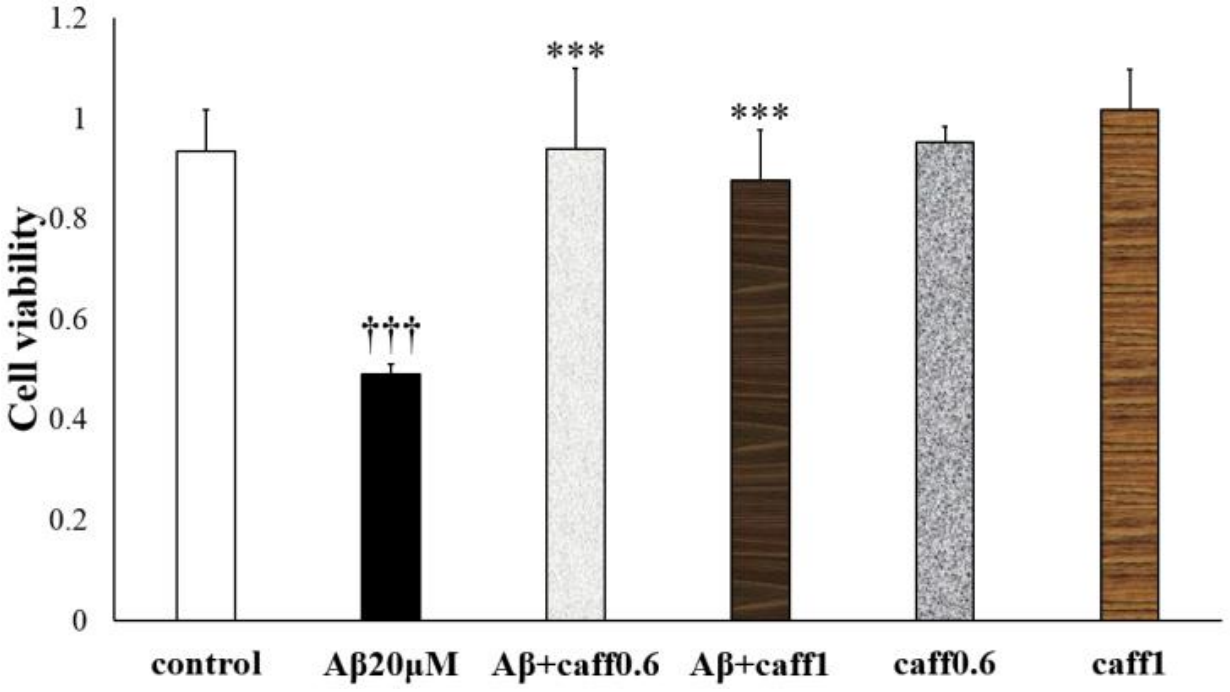

Fig.1. The neurprotective effects of caffeine against beta-amyloid (Aβ)-induced neurotoxicity in SHSY5Y cells. Cell viability was measured via the MTT test. Data were analyzed by one-way analysis of variance (ANOVA) test followed by LSD test. †††shows the significance lower than 0.001 compared to the control-treated group. ***shows the significance lower than 0.001 compare to the Aβ-treated group. Caff0.6: caffeine 0.6mM and caff1: caffeine 1mM.

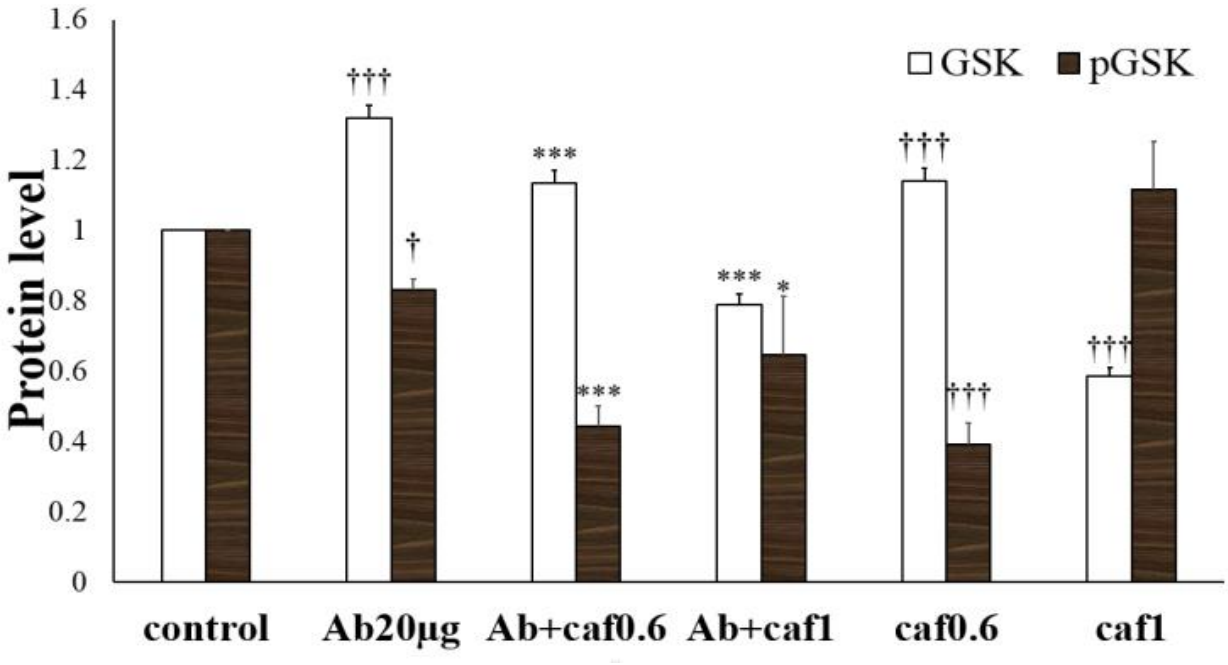

Fig.2. The effects of caffeine and beta amyloid (Aβ) on glucogen synthase kinase (GSK) and phosphorylated-GSK (pGSK) in SHSY5Y cells. The protein levels were measured by western blotting. Data were analyzed by one-way analysis of variance (ANOVA) test followed by LSD test. † & †††shows the significance lower than 0.05 and 0.001 compared to the control-treated group, respectively. * & ***shows the significance lower than 0.05 and 0.001 compare to the Aβ-treated group, respectively. Caff0.6: caffeine 0.6mM and caff1: caffeine 1mM.

decreased p-GSK-3β level (P<0.001) compared to the control group. In contrast, caffeine (1mM) without Aβ only decreased the GSK-3β level compared to the control-treated group (P<0.001).

Discussion

Our study showed that caffeine suppressed the neurotoxic effects of Aβ on SHSY5Y neuronal cells and reversed the Aβ effects on the GSK-3β and p-GSK-3β protein levels. Several reports have shown the neuroprotective effects of caffeine against neurodegenerative disorders. Caffeine protected the primary cerebellar neuronal cells against Aβ (Dall'Igna et al., 2003). Moreover, caffeine protected animals against Aβ neurotoxicity and prevented cognitive decline in animal models of AD (Cunha and Agostinho, 2010; Abreu et al., 2011). Caffeine also decreased Aβ level and suppressed plaque formation in transgenic mice (Chu et al., 2012).

The present study showed that caffeine inhibited Aβ effects on the GSK-3β and p-GSK-3β protein. Caffeine affects the GSK-3β signaling system in the peripheral tissues (Kim et al., 2016). In addition, caffeine has reduced free radicals and increased antioxidant enzymes in human neuroblastoma cells treated with Aβ and aluminum (Giunta et al., 2014). Previous studies have shown that increased oxidative stress and activation of GSK-3β may contribute to the neurotoxic effects of Aβ (Kamat et al., 2016). Therefore, the alleviation of oxidative stress and the reduction of GSK-3β may contribute to the neuroprotective effects of caffeine.

Caffeine in the absence of Aβ changed GSK-3β and p-GSK-3β protein levels. In contrast, in this condition caffeine exerted no effect on neuronal viability. The exact reason for this phenomenon is not completely clear. However, it is possible to assume that in non-stressful condition GSK-3β carries out normal cellular functions. In stressful conditions, this protein joins with apoptotic signaling and causes neuronal cell apoptosis. Therefore, the protein level of GSK-3β is not the sole factor responsible for neuronal cell apoptosis.

Conclusion

In conclusion, caffeine protected SHSY5Y neuroblastoma from Aβ-induced neurotoxicity and suppressed the effects of Aβ on the GSK-3β and p-GSK-3β protein levels. It is noteworthy that the GSK-3β protein level is not the single factor that

determines neuronal apoptosis.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interests.

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