Crocetin Prevents Amyloid β1-42-Induced Cell Death in Murine Hippocampal Cells

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ABSTRACT

Crocetin is an aglycon of carotenoid extracted by saffron stigmas (Crocus sativus L.) and known to have a potent anti-oxidative effect. Amyloid β (Aβ), hallmark of Alzheimer’s disease, is reported to have neurotoxicity partly via oxidative stress. In this study, we investigated the effect of crocetin on hippocampal HT22 cell death induced by Aβ1-42. Furthermore, to clarify the mechanism underlying the protective effects of crocetin against Aβ1-42-induced cell death, we measured reactive oxygen species (ROS) production by CM-H2DCFDA kit assay. Crocetin at 1 - 10 μM protected HT22 cells against Aβ1-42-induced neuronal cell death and decreased ROS production increased by Aβ1-42. These results that crocetin has the potent neuroprotective effect against Aβ1-42-induced cytotoxicity in hippocampal cells by attenuating oxidative stress, suggest that crocetin may provide a useful therapeutic strategy against Aβ-related disorders.

KEYWORDS

Alzheimer’s Disease; Amyloid β1-42; Crocetin; HT22; Oxidative Stress

1. Introduction

Alzheimer’s disease (AD) is a common neurodegenerative disorder, and amyloid β (Aβ) has been considered to have a critical role in the pathogenesis of AD [1]. AD is characterized by cognitive dysfunction and neuronal loss and these changes had been reported to occur as the result of various events, such as oxidative stress, neuroinflammation, and cholinergic deficits [2-4]. It is well known that the oxidative stress is involved in the mechanism of Aβ-induced neurotoxicity [5].

Crocetin, the aglycone of crocin carotenoids, is found in the saffron stigmas (Crocus sativus L.) and gardenia fruit (Gardenia jasminoides Ellis) [6,7] and they had been used for treatment of some diseases as traditional medicine [8]. Furthermore, crocetin has various pharmacological effects, such as anti-cancer [9], hepatoprotective [10], anti-oxidative [11], anti-inflammatory [8], and neuroprotective effects [12]. Crocetin has been reported to inhibit Aβ fibrillization and stabilize Aβ oligomers [13]. We recently reported that crocetin prevents retinal damage induced by H2O2, tunicamycin, and N-methyl-D-aspartate (NMDA) [14,15]; saffron extracts have been reported to be effective on memory deficit on mild AD patient [16,17]. However, there was no report on protective effects of crocetin in hippocampal neuronal cells having been examined.

In the present study, therefore, we investigated the effects of crocetin against Aβ1-42-induced cell death in HT22 mouse derived hippocampal cells.

2. Material and Methods

2.1. Materials

Aβ1-42 peptide (Abcam, Cambridge, UK) was dissolved in dimethyl sulfoxide (DMSO), then diluted in phosphate...
buffered saline (PBS; pH 7.4) according to manufacturer’s data sheets. Oligomers/fibrils of Aβ1-42 was achieved by incubation of 2 mM stock solution at 37°C for 2 h. Crocetin was obtained from Riken Vitamin Co., Ltd. (Tokyo, Japan).

2.2. Cell Cultures

Mouse hippocampal HT22 cells were gifted by Yoko Hirata Ph.D. (Gifu University, Japan). Cells were maintained in Dulbecco’s modified Eagle’s medium (D-MEM; Nacalai tesque, Kyoto, Japan) containing 10% fetal bovine serum (FBS), 100 units/mL penicillin (Meiji Seika Kaisha Ltd., Tokyo, Japan), and 100 μg/mL streptomycin (Meiji Seika) in a humidified atmosphere of 95% air and 5% CO2; at 37°C. Cells were passaged by trypsinization every 2 or 3 days, and maintained in a 10 cm dish (BD Biosciences, Franklin Lakes, NJ, USA).

2.3. Cell Death Assay

HT22 cells were seeded at 1 × 10³ cells per well into 96-well plates (BD Biosciences), then incubated for 24 h at 37°C in a humidified atmosphere of 95% air and 5% CO2. The entire medium was then replaced with fresh medium containing 1% FBS. Then, 2 μM of Aβ with or without 0.1 - 10 μM of crocetin, and N-acetyl-L-cysteine (NAC; Wako, Osaka, Japan) were added and incubated for 48 h at 37°C. NAC, which is well known to have a potent anti-oxidant effect, was used as a positive control in this study. Crocetin and NAC were dissolved in PBS containing 1% DMSO. After 46 h incubation, the medium was replaced with fresh medium containing 1% FBS, then the cells viability was measured by using CCK-8 kit (Dojindo, Kumamoto, Japan).

Nuclear staining assays were carried out after 48 h incubation. Cell death was assessed by combination staining with Hoechst 33342 (indication of alive and dead cells) and PI (indication of dead cells) (Figure 1(b)). Compared with the control group, Aβ1-42 induced neuronal cell death in a concentration-dependent manner (data not shown). Compared with the control group, Aβ1-42 at 2 μM significantly increased the percentage of dead cells. Treatment with crocetin at 3 μM protected HT22 cells against H2O2-induced cell death in a concentration-dependent manner (Figure 1(c)).

2.4. CM-H2DCFDA Kit Assay

The end of the culture period, HT22 cells were exposed to 10 μM 5-(and-6)-chloromethyl-2’, 7’-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H2DCFDA) (Invitrogen Life Technologies, Carlsbad, CA, USA) for 1 h. Fluorescence was measured by using a Varioskan flash 2.4 microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) at the excitation/emission wavelengths of 485/535 nm. The levels of reactive oxygen species (ROS) were calculated as percent increases compared with the control, and the control was normalized to 100% of the basal level.

2.5. Statistical Analyses

Data were presented as means ± S.E.M. Statistical comparisons were made using a two-tailed t-test or one-way ANOVA followed by Dunnett’s test, P < 0.05 being considered to indicate a statistical significance.

3. Result

3.1. Crocetin Protects HT22 Cells against H2O2-Induced Cell Death

At first, we evaluated the effect of crocetin against H2O2-induced cell death by using combination staining with Hoechst 33342 (indication of alive and dead cells) and PI (indication of dead cells) (Figure 1(b)). Compared with the control group, H2O2 (200 μM) significantly increased the percentage of dead cells. Treatment with crocetin at 3 μM protected HT22 cells against H2O2-induced cell death in a concentration-dependent manner (Figure 1(c)).

3.2. Crocetin Protects HT22 Cells against Aβ1-42-Induced Cell Death

Next, we evaluated the effect of crocetin against Aβ1-42-induced cell death by using nuclear staining (Figure 2(a)). Aβ1-42 treatment at 0.2 to 20 μM induced neuronal cell death in a concentration-dependent manner (data not shown). Compared with the control group, Aβ1-42 at 2 μM significantly increased the percentage of dead cells. Treatment with crocetin at 1 to 10 μM protected HT22 cells against Aβ1-42-induced cell death (Figure 2(b)).

We also evaluated the effect of crocetin on cell viability on HT22 cells, by using CCK-8. Compared with vehicle control, Aβ1-42 at 2 μM significantly decreased the cell viability rate. Treatment with crocetin at 10 μM significantly reversed HT22 cells against Aβ1-42-induced decrease cell viability (Figure 2(c)).

3.3. Effect of Crocetin for ROS Production of HT22 Cells at Aβ1-42-Induced Cell Death

To clarify the mechanism underlying the protective effects of crocetin against Aβ1-42 (2 μM)-induced cell death,
we investigated the ROS production by CM-H2DCFDA kit assay. Treatment with Aβ1-42 at 2 μM significantly increased the ROS production, whereas treatment with crocetin at 1 to 10 μM decreased the ROS production induced by Aβ1-42 (Figure 3). NAC at 10 mM was used as a positive control, and reduced the Aβ1-42-induced increase in ROS by about 80%.

4. Discussion

In the present study, we investigated the neuroprotective effects of crocetin against Aβ1-42-induced neurotoxicity in murine hippocampal cells. Crocetin prevented Aβ1-42-induced cell death (Figure 1). Crocetin also reduced the ROS production induced by Aβ1-42 (Figure 3). These results suggest that crocetin could be a potential therapeutic agent for Alzheimer's disease.
murine HT22 hippocampal cells. Addition of Aβ1-42 peptide to HT22 cells induced cell death as well as a previous report [18]. Crocetin has been known to have potent anti-oxidant [19], anti-cancer [9], and anti-inflammation activities [8]. A number of researches show that oxidative stress involves in AD pathogenesis [20]. Hence, crocetin, a potent antioxidant, is considered to have potential of therapy for AD pathogenesis. As we predicted, crocetin showed neuroprotective effects against H2O2- and Aβ1-42-induced neuronal cell death.

Furthermore, crocetin at concentrations of 1 to 10 μM showed significantly the protective effect against Aβ1-42-induced neurotoxicity. In rat, crocetin distributed in plasma at 0.14 mM and in brain at about 40 μM [2]. Consequently, oxidative stress by ROS is considered the main pathway. A number of researches tend them attention to oxidative stress. However, the other mechanisms had been reported in partly. In previous reports, the protective effects of crocetin against various stresses in retina related to inhibition activity for caspase-3 and caspase-9 [14,15]. These results suggest that crocetin also inhibits caspase-3 and caspase-9 expression to protect the brain against cells apoptosis.

Crocetin or crocin (crocetin di-gentiobiose ester) has been reported to have the protective effects against various cognitive impairment models induced by ethanol, streptozotcin, scopolamine, aging, captured stress, or brain ischemia in vivo [27-31]. Taken together, these findings suggest that crocetin may be useful to prevent AD.

In conclusion, the present findings indicate that crocetin has neuroprotective properties against Aβ1-42-induced cytotoxicity in murine HT22 hippocampal neuronal cells, by attenuating oxidative stress.

REFERENCES


http://dx.doi.org/10.1016/0197-4580(96)00109-1


http://dx.doi.org/10.1016/S0140-6736(06)69113-7


http://dx.doi.org/10.1016/S0197-4580(01)00340-2

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Abbreviations

Aβ: Amyloid β;
AD: Alzheimer’s Disease;
CCK-8: Cell Counting Kit-8;
CNS: Central Nerve System;
D-MEM: Dulbecco’s Modified Eagle’s Medium;
DMSO: Dimethylsulfoxide;
FBS: Fetal Bovine Saline;
NAC: N-Acetyl-L-Cysteine;
NMDA: N-Methyl-D-Aspartate;
PBS: Phosphate Buffered Saline;
PI: Propidium Iodide;
ROS: Reactive Oxygen Species.