Research Paper: The Effect of Callistephin on Amyloid Beta-Induced Neurotoxicity in the Hippocampus of Male Rats

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Citation: Ghasemi Moravej F, Shahidi S, Mehdizadeh M, Soleimani Asl S. The Effect of Callistephin on Amyloid Beta-Induced Neurotoxicity in the Hippocampus of Male Rats. Anatomical Sciences. 2017; 14(2):73-78.

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Article info:
Received: 27 Dec. 2016
Accepted: 01 May. 2017
Available Online: 01 Jul. 2017

Key Words:
Alzheimer's disease,
Neurogenesis, Anthocyanins

ABSTRACT

Introduction: Oxidative stress plays a key role in the pathophysiology of the Alzheimer's disease and it seems that antioxidants may slow the progress of the disease. The current study aimed at investigating the possible protective effects of callistephin (a natural flavonoid) against amyloid β (Aβ)-induced neurogenesis deficits in rats.

Methods: Adult Wistar male rats in the current study were treated with intrahippocampal Aβ as well as intraperitoneal callistephin injection. The day after the last administration, neuroD expression was assessed using an immunohistochemistry method. Finally, data were analyzed with SPSS using the one-way ANOVA test.

Results: Results of the current study showed that Aβ significantly decreased the expression of neuroD and callistephin could attenuate these changes.

Conclusion: In conclusion, results of the study suggested that callistephin can improve Aβ-induced neurogenesis changes in rats.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia in elderly adults characterized by progressive cell loss, apoptosis, and synaptic and memory defect [1, 2]. Deposition of Amyloid Beta (Aβ) and hyperphosphorylation of tau protein are the most important pathogenesis of AD [3]. Evi-

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dence documented that oxidative stress with consequent neurogenesis alteration in the hippocampus might also contribute in the pathogenesis of AD [4]. Neurogenesis occurs throughout life in the subventricular zone of the lateral ventricle and subgranular zone of the dentate gyrus of hippocampus [5].

NeuroD (neurogenic differentiation) as a biomarker of neurogenesis, plays a critical role in the neurogenesis that is downregulated following the incidence of neurodegenerative diseases [6, 7]. Accumulating evidence indicated that the use of antioxidant nutrients could improve the detrimental consequence of Aβ [8] and considered to be a promising approach toward neuroprotection against neurotoxic agents [9]. Callistephin, also known as pelargonidin, is a flavonoid with protective activities in diabetic neuropathic hyperalgesia and hemi-parkinsonism [10, 11]. It is found in pomegranate, strawberries, purple corn, berry skins of Cabernet Sauvignon and Pinot noir grapes. As mentioned above, Aβ deposition leads to stress oxidative and neurogenesis deficit in the hippocampus. Thus, the current study aimed at investigating the effect of callistephin, as an antioxidant, on the treatment of Aβ-induced neurotoxicity.

2. Materials and Methods

Aβ (1-42) was purchased from Tocris Company (Bristol, United Kingdom) and the materials required for Immunohistochemistry (IHC) assays were obtained from Abcma Company (Abcam, Cambridge, UK). Twenty-five adult male Wistar rats (weighed 250-300 g) were obtained from the animal center of Hamadan University of Medical Sciences, Hamadan, Iran (HUMS) and housed at 21±2°C with 50%±5% humidity controlled colony room under 12:12 hours light/dark cycle with ad libitum access to food and water. All animal care and experiments were approved by the Veterinary Ethics Committee of HUMS (approval code: 93/105/610) and were performed according to the National Institute of Health Guide for care and use of laboratory animals.

The rats were randomly classified into 5 following experimental groups: 1. Intact control; 2. Sham operated; 3. Aβ group, which received bilaterally Intrahippocampal injection (IHP) of Aβ (1-42); 4. Callistephin-treated Aβ, which received a single intraperitoneal (IP) injection of 3 mg/kg callistephin 2 weeks after Aβ injection [11]; 5. Callistephin, which received a single IP injection of 3 mg/kg callistephin.

Intrahippocampal Aβ 1-42 injection

Aβ (1-42) was dissolved in distilled water at the concentration of 1 µg/µL. Briefly, IHP Aβ (5 µg) injection was performed under ketamine (100 mg/kg) and xylazine (10 mg/kg) anesthesia using a stereotaxic apparatus (coordinates anterior-posterior=-3.6; lateral=±2.3; and dorsal-ventral=3 mm) [8]. The sham operated group received vehicle solution.

Immunohistochemistry analysis

The rats were anesthetized with ketamine and xylazine, and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH=7.3). The brains were removed and post-fixed in the same fixation solution. The tissue samples were processed in ascending series of ethanol, xylene, and paraffin. Next, 10-µm coronal sections were prepared by microtome, deparaffinized, and dehydrated. Then, the IHC staining was performed to detect the distribution of NeuroD in the hippocampus according to manufacturer's instruction.

In brief, after antigen retrieval in sodium citrate buffer by microwave in 5 minutes and blocking in 10% normal serum with 1% Bovine Serum Albumin (BSA) in TBS (Tris-buffered saline) for 2 hours, the sections were incubated overnight with a rabbit anti-NeuroD1 antibody (1:100, Abcam, Cambridge, UK) at 4°C and HRP-conjugated anti-rabbit IgG used as secondary antibody (1:500, Abcam, Cambridge, UK) for 1 hour. Then, the slides were incubated with DAB (3,3'-diaminobenzidine) (Abcam, Cambridge, UK) for 20 minutes and counterstained using hematoxylin. Finally, images were provided from slides using a digital camera attached to a light microscope. The number of the dark brown cells expressing neuroD was counted.

Data analysis

Data were expressed as mean±SEM and analyzed using one-way ANOVA followed by the Tukey multiple comparison test. $P \leq 0.05$ was considered the level of significance.

3. Results

Figure 1 shows the schematic representation of the coronal sections of the Cornu Ammonis (CA) and Dentate Gyrus (DG) of the hippocampus. For each animal, the average number of dark brown cells representing the expression of neuroD1 was obtained by counting 5 serial sections (Figure 2, A-E). Mean±SEM of the brown cells in the DG are given in Figure 3. Results of the current study showed that Aβ injection in hippocampus significantly reduced the number of NeuroD positive cells in the DG, compared with the control and sham-operated groups ($P<0.001$, Figure 3). Administration of cal-

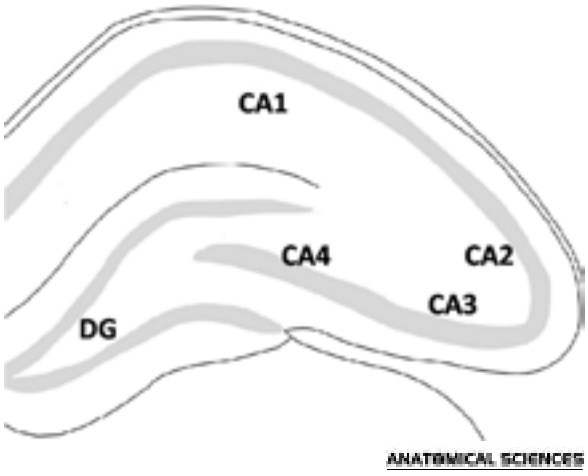

Figure 1. Schematic view of the structure of the hippocampus

listephin in order to Aβ treatment led to increase in NeuroD positive cells compared with that of the Aβ Group ($P<0.05$). Furthermore, there was a significant difference between the callistephin and Aβ Groups ($P<0.001$).

4. Discussion

Results of the current study showed that the callistophin attenuated Aβ-induced deficit in neurogenesis by increasing the number of NeuroD positive cells. Evidence showed that deposition of Aβ in the hippocampus can destroy neurons and results in cognitive deficits [12]. There is a relation between Aβ and oxidative stress [13]. Oxidative stress reflects an imbalance between the production of Reactive Oxygen Species (ROS) and endogenous antioxidant defenses (a biological system that can detoxify the ROS) in order to repair the resulting damage [14]. In this regard, it is shown that Aβ administration induces oxidative stress, inhibits NMDA (N-methyl-D-aspartic acid or N-methyl-D-aspartate)-receptor associated synaptic transmission [15], and may accelerate mitochondrial permeability transition opening [16].

Although there are many chemical and herbal therapies to decline the AD neurotoxicity, no permanent treatment for AD is developed. Use of anthocyanin as a flavonoid in the human diet is one of the most common therapeutic strategies for treatment or restoring of neurodegenerative diseases [8, 17]. Callistephin is an anthocyanin that can cross the brain blood barrier [18] and show protective effects in the Parkinson disease [10].

Results of the current study demonstrated that administration of callistephin led to an increase in neurogenesis in Aβ-treated rats that is confirmed by other studies. Similarly, it is reported that anthocyanin plays beneficial role in the treatment of mental disorders and is able to increase cell density in the hippocampus of rats exposed to chronic stress [19]. In another study, anthocyanin attenuated kainic acid-induced ROS accumulation, activa-

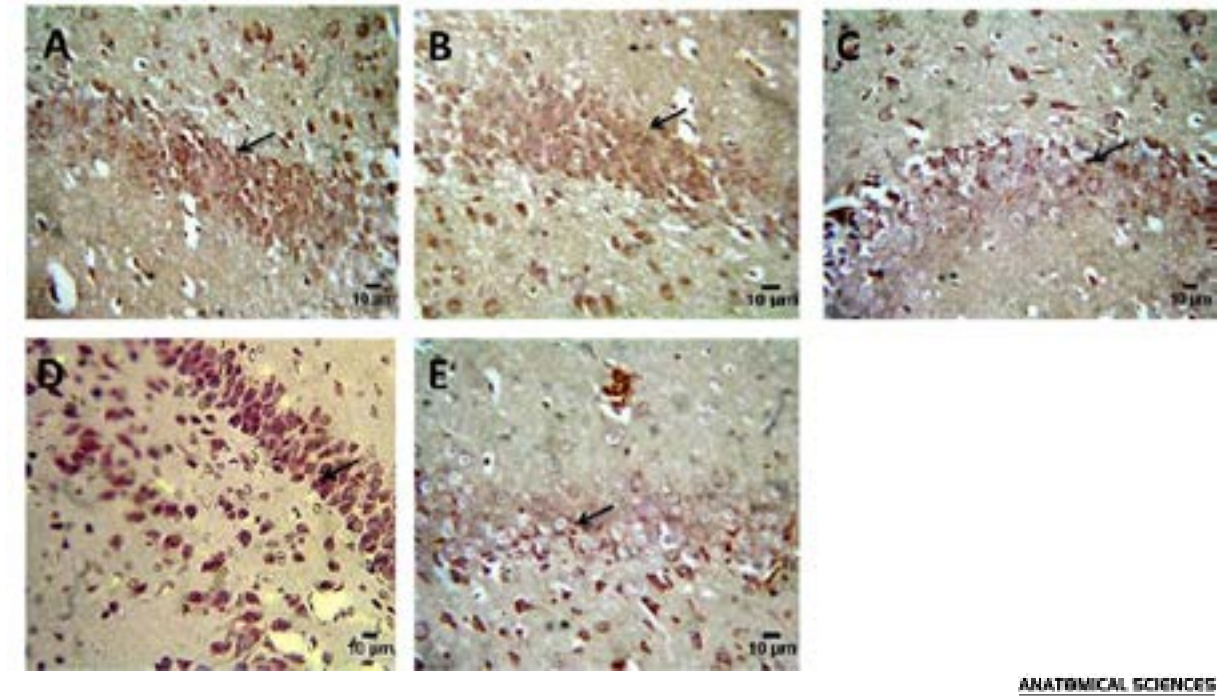

Figure 2. Immunohistochemical staining of hippocampus in the control (A); Callistephin (B); Sham (C); Beta-amyloid (D); and Beta-Amyloid and callistephin groups (E)
Arrows show NeuroD1 positive cells.

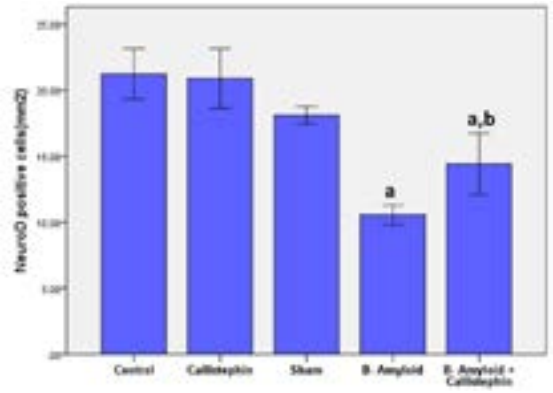

Figure 3: Mean±SEM of the NeuroD1 positive cells
a: P<0.001 vs. the control, sham, and callistephin groups; b: P<0.05 vs. the beta- amyloid group

tion of AMPK (5'-adenosine monophosphate-activated protein kinase), and increase in percentage of apoptotic cells in the mouse hippocampal cell line [20].

Consistent to the current results, Skemiene et al. showed that callistephin decreased the apoptosis in the ischemia model [21]. It seems that callistephin administration leads to restore the Superoxide Dismutase (SOD) and catalase in diabetic rats [11], and decrease neuronal damage and loss [22] via enhancement of the antioxidant defense system. It seems that callistephin inhibits ROS generation and subsequently suppresses the inhibitory effects of Aβ on neurogenesis, and it is maybe useful to protect against Aβ-induced neurotoxicity.

Acknowledgments

The research was supported by a grant from Hamedan University of Medical Sciences, Hamadan, Iran (No. 9312126432).

Conflict of Interest

The authors declared no conflicts of interest.

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