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Original Article

Anthocyanins attenuate post-operative cognitive dysfunction via upregulation of SIRT3 in diabetes mellitus mice

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Abstract: Objective: The study aimed to determine the protective effect of anthocyanins on post-operative cognitive dysfunction via upregulation of SIRT3 in diabetes mellitus mice. Methods: A total of 96 healthy adult male BALB/c mice (30-50g) were randomly divided into 4 groups (n=24 each). These included the following groups: the control group with no surgery, the sham group of type 2 diabetic mice established first, followed by splenectomy, and the anthocyanin group as a model of type 2 diabetic mice. Mice were continuously gavaged with anthocyanins 100 mg/kg/d for 2 weeks prior to splenectomy. An additional SIRT3-knockout group was established as the diabetic mice model, SIRT3 was knocked out prior to the same administration of anthocyanins with anthocyanin group. To detect spatial memory and learning abilities, 8 mice in each group were randomly chosen for Morris water-maze (MWM) test on day 8, 10, 14 after splenectomy to determine the time duration of escape latency, total quadrant crossing, and original quadrant. Levels of TNF-α and IL-1β in hippocampuses were detected by ELISA kits. Results: 1. Morris Water-Maze test results. At day 10 and 14, mice in the Sham group compared to the Control group, the escape latency was significantly prolonged, and total quadrant crossing and time in original quadrant were shortened ($P < 0.05$). When mice in the Anthocyanin group were compared to the Sham group, the escape latency was significantly shortened, and total quadrant crossing and time in original quadrant were prolonged. Mice in the SIRT3-knockout group was compared to the Anthocyanin and Control group, and the escape latency was significantly prolonged, and total quadrant crossing and time in original quadrant were shortened ($P < 0.05$). 2. Pro-inflammatory cytokines in the hippocampus of mice. At day 8, 10 and 14, mice in the Sham group was compared to the Control group, TNF-α and IL-1β were significantly increased ($P < 0.05$). When mice in the Anthocyanin group were compared to the Sham group, TNF-α and IL-1β were significantly decreased. When mice in the SIRT3-knockout group were compared to the Anthocyanin and Control group, TNF-α and IL-1β were significantly increased ($P < 0.05$). Conclusions: Anthocyanins may play a protective effect of post-operative cognitive dysfunction via upregulation of SIRT3 in diabetes mellitus mice.

Keywords: Anthocyanins, POCD, SIRT3, diabetes mellitus

Introduction

Post-operative cognitive dysfunction (POCD) is a complication of the central nervous system caused by anesthesia and/or surgery, mainly manifested as the decline of learning and memory ability, personality and social behavior ability [1]. POCD incidence overall in elders appears in the order of 10-38% [2-5] within the first 2 to 3 months and 3-24% at 6 to 12 months after major surgery [4, 6, 7]. However patients with diabetes are overall at 26% increased risk of POCD compared with diabetes-free patients [8]. Although the molecular mechanism of POCD has not been fully illustrated, neuroinflammation was considered to be closely associated with POCD after general anesthesia of surgery in rats. It has been suggested that the release of pro-inflammatory cytokines increased after surgery, including TNF-α and IL-1β, which could trigger a wide spectrum of neuroinflammations in the brain and increase POCD [8-10]. Several studies recently demonstrated that anthocyanins could reduced cogni-

tive dysfunction of Alzheimer's disease from a neuroinflammatory and oxidative stress perspective [11-13], and sirtuins 3 (SIRT3) is closely associated with the regulation of neuroinflammatory and oxidative stress perspective [14-16]. However, the effect of anthocyanins in POCD impairment in diabetes mellitus (DM) populations and the function of SIRT3 in POCD are without robust evidence. This study was aim to illustrate the protective effect of anthocyanins on POCD in DM mice with possible SIRT3 pathway.

Materials and methods

Animals and groups

Our animal protocol was approved by the Animal Care and Use Committee at Southwest Medical University in accordance with the requirements of the Chinese Animal Care Committee. All of the animals used in this study were in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

A total of 96 healthy adult male BALB/c mice (30-50 g) were randomly divided into 4 groups (n=24 each). Control group: no surgery, Sham group: the model of type 2 diabetic mice was established first (streptozyme 20-40 mg/kg/day was administered by tail vein for 5 days, and hyperlipidemia was kept for several days. After 5 days, modeling success was confirmed when fasting blood glucose > 10 mmol/L or random blood glucose > 16.7 mmol/L), followed by splenectomy. Anthocyanin group: the model of type 2 diabetic mice was first established, and then they were continuously gavaged with purple sweet potato anthocyanin (PSPA) 100 mg/kg/d for 2 weeks prior to splenectomy. SIRT3-knockout group: same establish of diabetic mice model, SIRT3 was knocked out prior to the same administration of anthocyanins with Anthocyanin group.

PSPA preparation

The PSP cultivars Anhui Zi was obtained from the Academy of Agricultural Sciences in Xuzhou (Jiangsu, China). Based on previously described methods [17], 5 to 8 mm PSP slices were dried under the condition of 50°C (moisture content in dry products < 12%). Subsequently, the dry PSP slices were made into powder.

Morris water-maze (MWM) test

MWM has become the most frequently used one as it can circumvent sensory and motor defects which may interfere with experimental results, thus can more accurately reflect spatial memory of mice. In MWM, navigation sessions can evaluate the learning and memory ability, while space exploration session can measure the spatial association ability, recall ability, and explore ability [19].

In each group, 8 mice were randomly chosen for MWM test on day 8, 10, 14 after splenectomy. In order to detect spatial memory and learning abilities, first, navigation training sessions were performed, in which mice facing the wall of the pool were released into the water from one of four quadrants. A platform was placed in the center of test field. Mice were allowed to locate the platform and land on it within 60 seconds. If the mouse failed to do it within 60 seconds, they were picked up and maintained on the platform. Those mice were assigned as having a 60 second escape latency. After landing on the platform, each mouse was kept on it for 15 seconds to strengthen the memory of the platform. This session was conducted four times each day for each mouse, with the entry from one of the four quadrants each time. The escape latency was recorded and averaged. After the navigation training, the platform was removed. The test session began from the contralateral quadrant of original platform quadrant with mice facing the wall of the pool. The time duration in the original platform quadrant and the frequency of crossing were recorded and calculated.

Enzyme-linked immunosorbent assay (ELISA)

After the MWM test, mice were sacrificed; hippocampuses were harvested, grinded with homogenizer and prepared for 10% homogenates by ultrasonic. The homogenates were centrifuged at 2500 r/min at 4°C for 10 minutes. Levels of TNF-α and IL-1β were detected by ELISA kits (Rapidbio, California, USA) according to the manufacturer's instructions.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) 20.0 (IBM). Comparisons between groups were conducted using two-way analysis of variance (ANOVA)

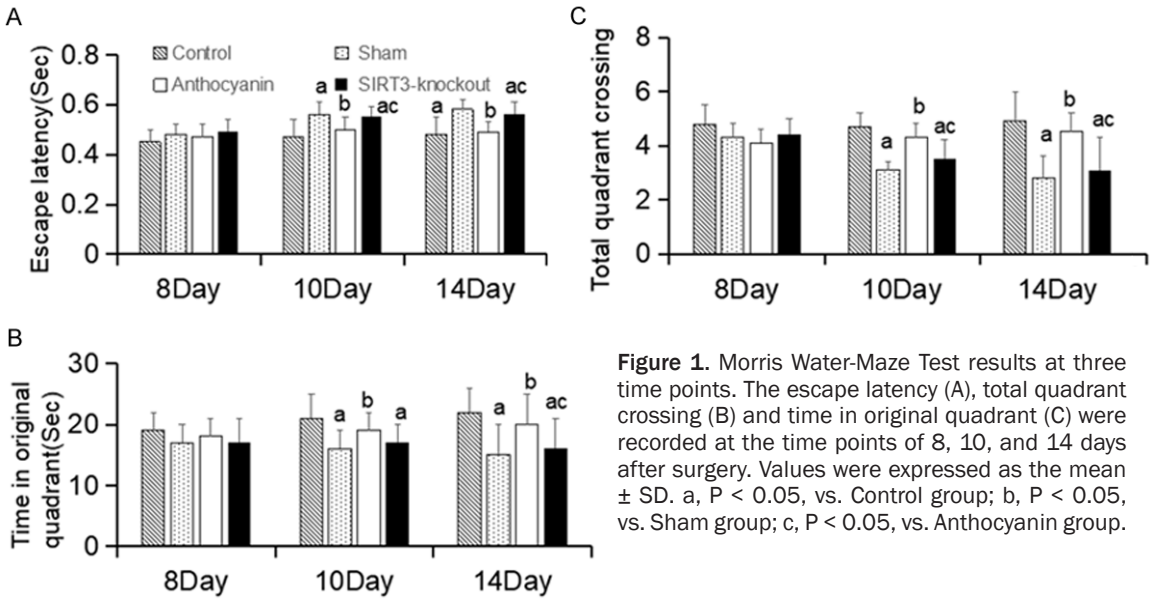

Figure 1. Morris Water-Maze Test results at three time points. The escape latency (A), total quadrant crossing (B) and time in original quadrant (C) were recorded at the time points of 8, 10, and 14 days after surgery. Values were expressed as the mean ± SD. a, P < 0.05, vs. Control group; b, P < 0.05, vs. Sham group; c, P < 0.05, vs. Anthocyanin group.

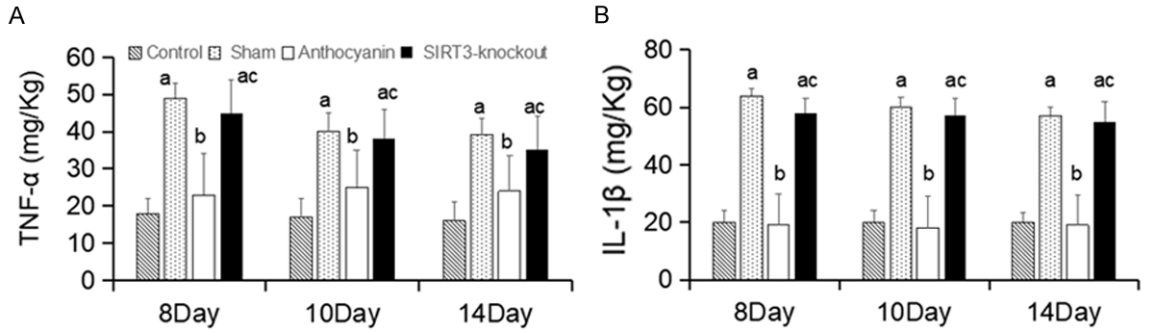

Figure 2. Pro-inflammatory cytokines in the hippocampus of mice. Levels of TNF-α (A) and IL-1β (B) in the hippocampuses were detected by ELISA kits. Values were expressed as the Mean ± SD. a, P < 0.05, vs. Control group; b, P < 0.05, vs. Sham group; c, P < 0.05, vs. Anthocyanin group.

plus post hoc Bonferroni comparison. Unless otherwise noted, data are presented as the mean (SD). Significance was defined as a two-sided *P*-value < 0.05.

Results

Morris Water-Maze test results

At day 10 and 14, mice in the Sham group were compared to the Control group and the escape latency was significantly prolonged, and total quadrant crossing and time in original quadrant were shortened (P < 0.05). Mice in the Anthocyanin group was compared to the Sham group and the escape latency was significantly shortened, and total quadrant crossing and time in original quadran were prolonged. Mice in the SIRT3-knockout group were compared to the Anthocyanin and Control group, and the escape latency was significantly prolonged, and total quadrant crossing and time in original quadrant were shortened (P < 0.05) (**Figure 1**).

Pro-inflammatory cytokines in the hippocampus of mice

At day 8, 10, and 14, mice in the Sham group were compared to the Control group, TNF-αand IL-1β were significantly increased (P < 0.05) and mice in the Anthocyanin group were compared to the Sham group, and TNF-α and IL-1β were significantly decreased. Mice in the SIRT3-knockout group were compared to the Anthocyanin and Control group, where TNF-α and IL-1β were significantly increased (P < 0.05) (**Figure 2**).

Discussion

Our aim was to test the hypothesis that anthocyanins may have a protective effect on POCD in DM mice after surgery via down regulation of SIRT3. The goal of this study was to examine the effects of anthocyanins on POCD effects in DM mice and determine the cognitive functions of mice in the anthocyanin group. Mice were significantly improved at day 8, day 10, and day 14 after surgery in comparison to the Sham group. These results showed that the anthocyanins may alleviate long-term POCD symptom in DM mice. Furthermore, after administration of anti-SIRT3, there was no significant difference of cognitive functions at these three time points between the anthocyanin group and the anti-SIRT3 group, which showed that anthocyanins might play a protective role against POCD in DM mice after surgery via up regulation of SIRT3. There are a few studies showing that anthocyanins may reduce the incidence of POCD via decrease of neuroinflammation and oxidative stress [19, 20].

How anthocyanins play a protective effect on POCD in DM mice after surgery was a focus of this study. Expression of IL-1β, TNF-α were up regulated in the Sham group in which there was the highest incidence of POCD among the three groups, and an increase of IL-1β and TNF-α was alleviated after administration of anthocyanins and showed a lower incidence of POCD compared with the Sham group. TNF-α can activate neural glial cells and has pivotal roles in neuromodulation [21, 22]. Recently, Sun D et al. demonstrated that administration of dobutamine hydrochloride alleviated development of early POCD in aged patients via inhibiting the release of TNF-α [23]. IL-1β also plays an important role in the development of POCD, Barrientos RM demonstrated that the learning and memory in rat could be impaired by injecting IL-1β into the hippocampus [24]. Symptoms of POCD could be alleviated by the knockout of IL-1β receptor in mice [22].

The role of SIRT3 in the regulation of TNF-α and IL-1β pretreated with anthocyanins in Sirt3 blocked DM mice was also examined. Inflammatory medium (TNF-α, IL-1β) and the incidence of POCD was significantly higher in Anti-Sirt3 than in the Anthocyanin group. Sirt3 may play an important role in the development of POCD and may regulate the release of inflammatory mediators. The close relationship between SIRT3 and inflammatory mediators [25-27], and Li, JJ et al. [28] further found that SIRT3 could mediate the expression of TNF-α and IL-1β, which were closely associated with POCD.

There are also limitations of this study. There are quite a few studies about the association between inflammatory mediators and POCD, therefore we still have no robust evidence about the association between anthocyanins and POCD, SIRT3, and POCD. Further research is needed in the future.

In conclusion, the present study demonstrated the protective effect of anthocyanins for splenectomy-induced POCD in DM mice, possibly via up regulation of the activity of SIRT3, and eventually, by suppressing neuro-inflammation in the brain.

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Disclosure of conflict of interest

None.

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