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D-4F decreases the expression of Aβ protein through up-regulating long non coding RNA sirt1-as in SAMP8 mice

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ABSTRACT

Background and objective: Cholesterol plays key roles on (Aβ) metabolism and production. D-4F is the apolipoprotein A-I mimetic peptide which has been revealed a critical role in regulation cholesterol. We aimed at identifying the effects of D-4F on Aβ production in SAMP8 and the underlying mechanisms. Methods: SAMP8 mice (n = 15) were randomized into three groups for treatment with D-4F given in drinking water: high-dose group (0.5 mg/ml), low-dose group (0.3 mg/ml) and control group (just drinking water). The heart, kidney, liver and brain were obtained from SAMP8 (9 of them included in the analysis). The long non-coding RNA sirt1-as was measured in all tissues. The immunohistochemistry, western blot qRT-PCR were performed to determine the sirt1-as and the relevant proteins or RNAs levels. Results: After treated with D-4F, the sirt1-as has been significantly upregulated in brain, rather than heart, kidney or liver. Specially, sirt1-as was significantly up-regulated by high dose of D-4F in the hippocampus area ($p = 0.007$) compared with control group. Further analysis revealed that D-4F up-regulates the expression of SIRT1. We also found that D-4F treatment significantly increased the reverse cholesterol transport related proteins liver X receptor α (LXRα) and ATP-binding cassette transporter A1 (ABCA1, $p < 0.05$). Finally, the amyloid β-protein (Aβ protein) was statistically lower than that in the control group ($p < 0.05$). Conclusion: Our observation indicated that D-4F decreases the expression of Aβ protein through up-regulating long non coding RNA sirt1-as and its downstream proteins which may involve in reverse cholesterol transport.

1. Introduction

Alzheimer's disease (AD) has been demonstrated as the most common and hazardous dementia in the worldwide in aging populations (Brody, 2011). It has also indicated that the AD is caused by disequilibrium between amyloid β-protein (Aβ protein) production and clearance resulting in Aβ accumulation in brain. The Alzheimer's disease is characterized by two hallmark lesions, Aβ containing plaques and neuro fibrillary tangles (Brody, 2011). The Aβ deposition theory has been widely accepted as a well-established pathophysiological change in brain of AD patients (Reiman, 2016). To increase the clearance of Aβ or decrease the production of Aβ is a classic strategy to prevention AD in experiments. Furthermore, an essential finding that the cholesterol plays key roles on Aβ metabolism has been indicated in the previous studies. It has been demonstrated that cholesterol in the brain participates in a number of interdependent metabolism processes of Aβ such as the synthesis, neurotoxicity and clearance (Brody, 2011).

Based on the cholesterol-Aβ theory, the precise mechanisms basic and clinical researches have been performed. Up-regulating high density lipoprotein cholesterol (HDL-C) or lowering the low density lipoprotein cholesterol (LDL-C) may be benefic for patients with AD. The apolipoprotein A-I mimetic peptide D-4F has been revealed the role in inhibiting the development of atherosclerosis via its roles in regulation cholesterol (Khera et al., 2011). Redundant cholesterol acquired by extrahepatic tissues is delivered to the liver to excrete is called reverse cholesterol transport (RCT), which maintains whole body cholesterol homeostasis (Khera et al., 2011).

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The first step and rate limiting process of RCT is the cellular cholesterol transfer onto lipid-poor apolipoprotein A-I to form nascent HDL particles, and this process is mediated by ATP binding cassette A1 (ABCA1). The roles of D-4F in formation of HDL and improvements in high density lipoprotein (HDL) mediated cholesterol efflux and RCT from macrophages has been indicated (Cao et al., 2007; Plosch et al., 2007). Administration of D-4F has been proved to improve a number of pathological processes in different animal models

Furthermore, Silent Information Regulator Type 1 (SIRT1) is an essential NAD-dependent deacetylase with critical roles in cardiovascular disease and AD which were referred with cholesterol metabolism and Aβ synthesis via traditional beta-site APP-cleaving enzyme 1 (BACE1) pathway (Bonda, 2011; Godoy et al., 2014).

Though, the exact role of D-4F on RCT in has been identified in many other diseases and tissues. However, the roles of D-4F in AD have not been focused on in the past decades. Thus, based on the effectiveness and the mechanisms of D-4F in atherosclerosis and other diseases, we propose the hypothesis that D-4F may play a protective role in AD via reducing the Aβ in RCT mechanisms and performed the present study.

2. Methods

2.1. Materials

D-4F with the primary sequence Ac-DWFKAFYDKVAEKFKEAF-NH2 were synthesized by Shanghai Ketai Bio-Technique Co. Ltd. (Shanghai, China). The synthetic peptides purity process (typically > 98%) was carried out using analytical high performance liquid chromatography and it is confirmed using mass spectral analysis. Protease inhibitor cocktail was obtained from Roche Diagnostics GmbH (Mannheim, Germany). All antibodies were obtained from Abcam (Cambridge, UK). The BCA Protein Assay kit was purchased from Pierce Biotechnology (Rockford, IL, USA). The primers for sirt1-as, SIRT1, Aβ, ABCA1, BACE1 and LXRα were designed by using Primer 5.0 online tools and synthesized by Shanghai Invitrogen Ltd Co.

2.2. Animals and experimental protocols

The SAMP8 mice (n = 15) were provided by the Hua-Fukang Company and all of them were fed according to the standards for laboratory animals of China. The 26-week old SAMP8 mice were randomized into three groups for treatment with D-4F by drinking water: high-dose group (0.5 mg/ml), low-dose group (0.3 mg/ml) and control group (drinking water without D-4F) for 6 weeks prior to the examinations. Each mice consumed approximately 2.5 ml water/day, and the water or food consumption were similar among three groups.

All mice were fed with the D-4F for 8 weeks and they were anesthetized via intraperitoneal injection of sodium pentobarbitone (50 mg/kg). The venous blood samples were collected for further measurements of plasma biochemical makers. The brain, heart, kidney and liver were rapidly excised, immersed in ice-cold saline solution and blotted dry. The brain were dissected into five variant parts according the anatomy structure: frontal lobe, cerebral cortex and hippocampus etc. The samples were stored at −80 °C after liquid nitrogen freezing.

2.3. Examinations of the RNAs by using qRT-PCR

The heart, brain, liver and kidney levels of sirt1-as were determined by using a commercial kit for qRT-PCR. The RNA levels of SIRT1, LXRα, BACE1, ABCA1 and Aβ were also measured by using qRT-PCR. The procedure was as following: The populations were quantified from isolated DNA and RNA which is converted to cDNA using a qualified TaqMan PCR system utilizing the primer pairs. These amplification genes were analyzed with Light Cycler Software (Roche Applied Science).

2.4. Immunohistochemistry study

The Aβ protein was analyzed and located by using immunohistochemistry in SAMP8. The simplified processes of immunohistochemistry were list as following: Dry frozen tissue sections at 25 °C (room temperature) for two hours. Then fix the sections using 4% paraformaldehyde for 15 minutes. Wash the slides, block endogenous peroxidase via 30 minutes' incubation and block non-specific binding sites for 20 minutes. Incubate the slides overnight at 4 °C, with the appropriate antibody. Add avidin-biotin-peroxidase reagents, and reveal the peroxidase activity by incubating the slides. Dehydrate the slides with sequential ethanol washes of one minute each starting with 75%, then 80% and finally wash with a 100% ethanol. The seal slides and analyze using optical microscopy.

2.5. Western blot analysis

Proteins were extracted from brain tissues sample by using 200 μL of ice-cold buffer (pH 7.4) in the presence of phosphatase inhibitors. Protein concentrations were measured with the BCA protein assay kit. The lysates were separated by 10% sodium dodecyl-sulfate polyacrylamide electrophoresis and then it was transferred onto fluoride membranes. The membranes were also blocked using 5% non-fat milk powder in phosphate buffered saline for one hour at 25 °C and then they were incubated for an overnight with anti-SIRT1 (1:1,000), anti-BACE1 (1:1,000), anti-ABCA1 (1:1,500) and anti-LXRα (1:500). The membranes were then incubated for one hour with horseradish peroxidase-labeled anti-IgG (1:1000) at 37 °C. Finally, β-actin was used as a loading control. The results were expressed as fold increase compared with control.

2.6. Statistical analysis

The continuous variables in our study were expressed as mean ± SD. Their differences among groups were compared by one-way analysis of variance. The changes of the measured proteins and RNAs were calculated as folds compared with control or reference (β-actin) All analyses were conducted using SPSS software for Windows, version 19.0 (SPSS, Chicago, IL, USA). Statistical significance was at $P < 0.05$.

3. Results

3.1. Overall expression of sirt1-as in SAMP8 mice is regulated by D-4F feeding

The effect of D-4F on long non-coding RNA sirt1-as in various organs were revealed in Fig. 1(a–f) tested by qRT-PCR. After treated with D-4F, the sirt1-as has been significantly upregulated in brain (Fig. 1d–f), rather than heart, kidney or liver. Specially, sirt1-as was significantly up-regulated by high dose of D-4F in the hippocampus area (Fig. 1d, $p = 0.07$) compared with control group.

3.2. D-4F down-regulates the expression of Aβ in hippocampus

The SAMP8 mice is a classic AD model that expresses the AD's featured protein Aβ. Thus, we have also examined the protein Aβ

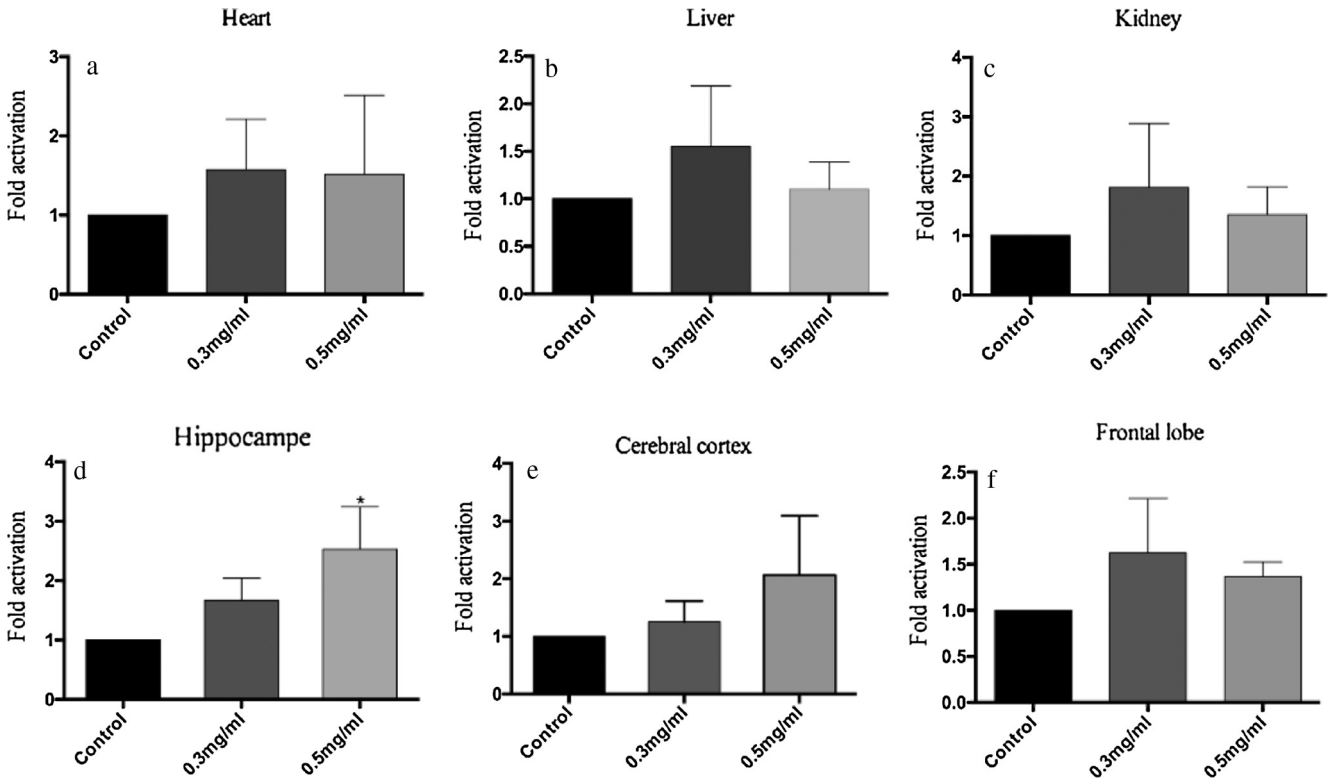

Fig. 1. Effect of D-4F on long non-coding RNA sirt1-as in SAMP8 mice. D-4F feeding increase the level of sirt1-as in hippocampus area instead of cerebral cortex or front lobe in SAMP8 mice. D-4F did not increase the level of sirt1-as in heart, kidney or liver (a-c, all p values > 0.05). *: $p < 0.05$ vs. control.

in various levels. Firstly, we detected the RNA level of protein Aβ using qRT-PCR, and found that it decreased significantly with a higher concentration of D-4F (Figs. 2 and 3).

3.3. D-4F up-regulates the expression of SIRT1

The target protein of sirt1-as, SIRT1, often plays various protective roles in aging, cardiovascular and cerebral-vascular diseases. Thus, we have detected the expression of SIRT1 in brain and found that the D-4F can significantly increase the expression of SIRT1 in RNA level and protein level (Fig. 4).

3.4. Effect of D-4F on the SIRT1 related proteins

The underling mechanisms that sirt1-as pathway have not been uncovered. We also examined the related target proteins which may be involved in RCT and regulated by SIRT1, which may participate in the pathological process of AD.

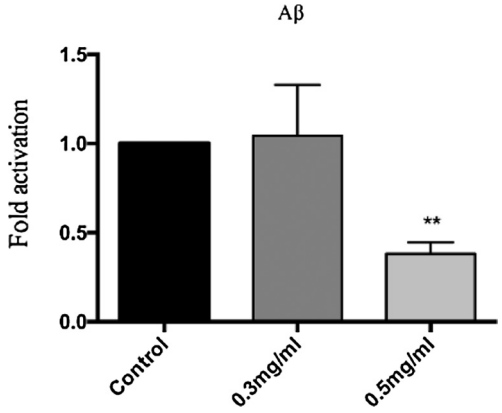

Fig. 2. D-4F feeding decrease the RNA of Aβ protein in SAMP8 mice, **: $p < 0.01$ vs. control.

The BACE1 was not affected by D-4F feeding while the LXRα and ABCA1were significantly upregulated by D-4F treatment. Ctr: control; H: high dose group, 0.3 mg/ml; L low dose group, 0.5 mg/ml. ∗: $p < 0.05$ vs. control; ∗∗: $p < 0.01$ vs. control.

The D-4F did not increase the BACE1 (left) level. However, it significantly increased the expressions of SIRT1 related LXRα (middle) and ABCA1 (right), especially in the high dose group ($p < 0.05$). Ctr: control; H: high dose group, 0.3 mg/ml; L low dose group, 0.5 mg/ml. a: Western blot image, b: Relevant qualified gray scale. ∗: $p < 0.05$ vs. control (Figs. 5 and 6).

4. Discussion

We have found that a relevant higher dose of D-4F decreases the expression of AD's featured protein Aβ and it up regulates the level of sirt1-as, SIRT1 and its related proteins rather than the traditional pathway via BACE1.

4.1. The sirt1-as was significantly increased in hippocampus area in SAMP8

It is the first time to investigate the effect of D-4F on the lncRNA sirt1-as which may up expresses SIRT1 in SAMP8 mice. The SAMP8 have been identified as a classic model of AD which expresses the Aβ protein and behaves similarly to the impaired cognitive of AD (Armbrecht et al., 2014). We have tested the level of sirt1-as (Wang et al., 2014) in major organs or tissues from SAMP8 mice. However, only the hippocampus area showed a significant increase of sirt1-as by feeding D-4F indicating that sirt1-as may participate the pathophysiological process of AD. Thus, we further examined its downstream protein SIRT1 and related proteins levels.

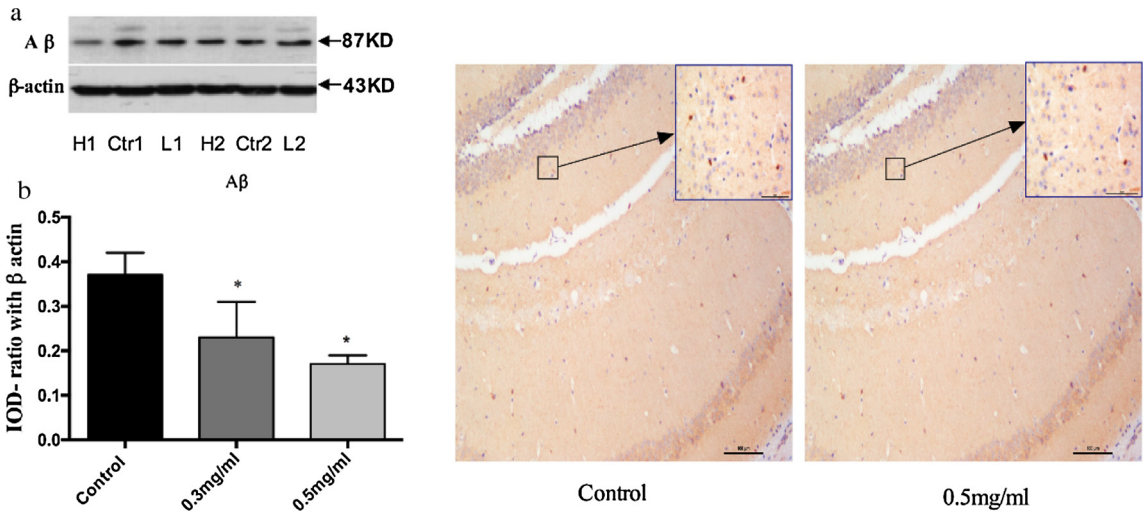

Fig. 3. The Western blot (left) and immunohistochemistry (right) results for Aβ protein. Ctr: control; H: high dose group, 0.3 mg/ml; L low dose group, 0.5 mg/ml. Integralopticaldensity (IOD) ratio to β actin was calculated as: IOD of Aβ/IOD of β actin. The Aβ was significantly lower in the high D-4F dose group and the low D-4F dose group than that in the control. *: $p < 0.05$ vs. control.

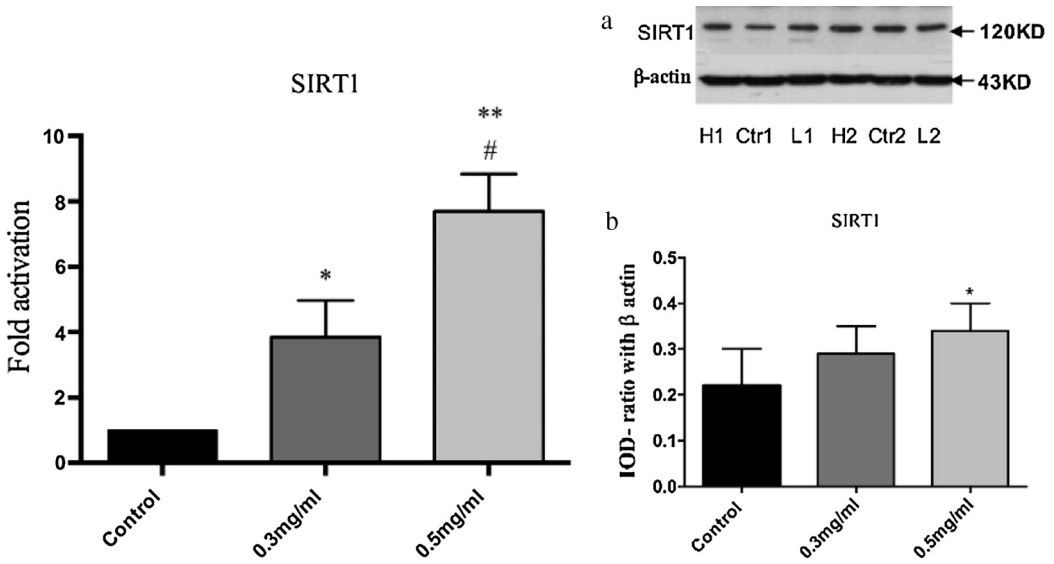

Fig. 4. D-4F feeding increased the RNA (left) and protein (right) of SIRT1 in SAMP8 mice. Ctr: control; H: high dose group, 0.3 mg/ml; L low dose group, 0.5 mg/ml. *: $p < 0.05$ vs. control. #: $p < 0.05$ vs. low dose group.

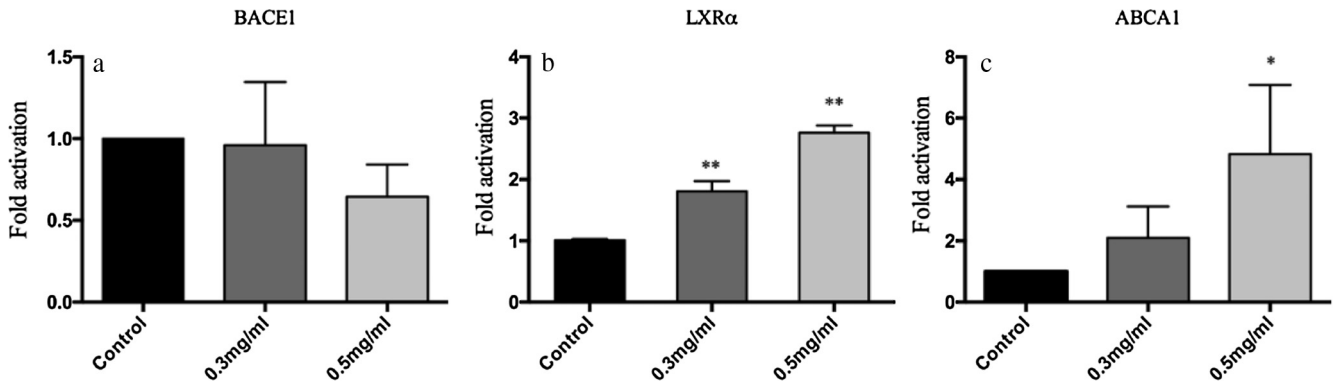

Fig. 5. The RNAs levels of BACE1, LXRα and ABCA1.

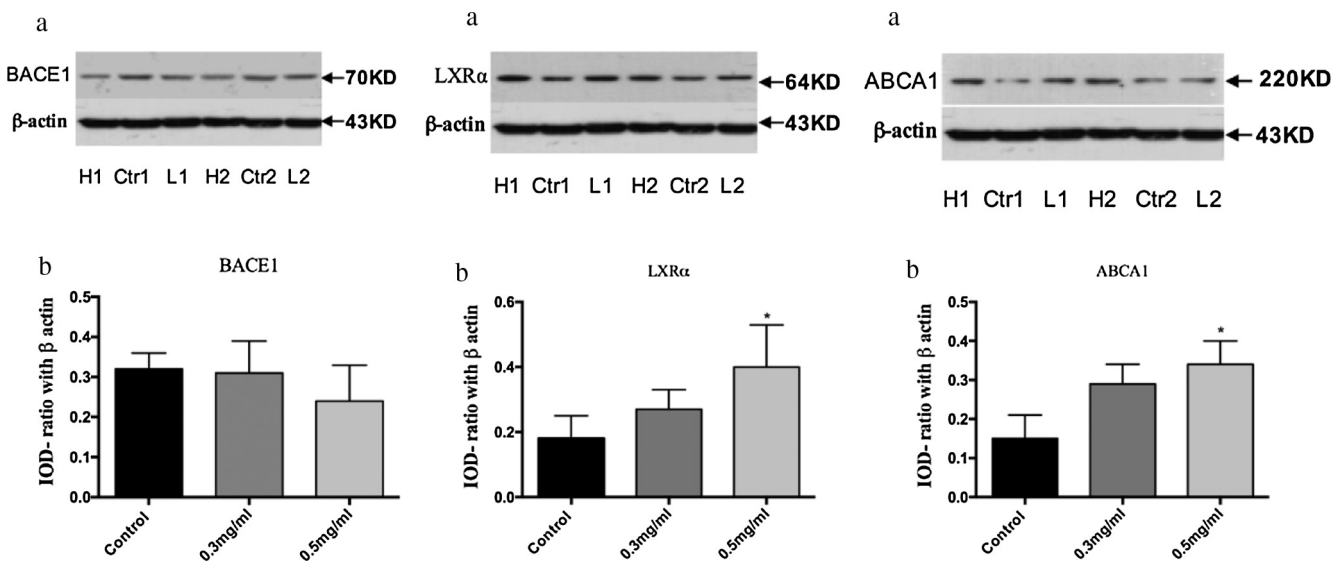

Fig. 6. D-4F feeding up-regulated the LXRα and ABCA1 proteins but BACE1.

4.2. D-4F feeding lowers the AD's featured protein Aβ in hippocampus area

We have focused our study of D-4F on the hippocampus area and tested the expression of Aβ after treated by D-4F in various doses orally. An exciting result showed that both doses of D-4F can down regulated the expression of Aβ protein both in protein and mRNA levels. This result indicated that D-4F may protect SAMP8 mice from AD which has not been widely uncovered in the previous studies (Armbrecht et al., 2015; Donmez,2012). Based on our above-mentioned results, we tried to investigate the potential roles of D-4F in protection of AD.

4.3. Potential mechanisms of D-4F down-regulates the expression of Aβ

We firstly measured the expression of an essential NAD-dependent deacetylase with critical roles in cardiovascular disease and AD, SIRT1, the target protein of sirt1-as, according to the observation that D-4F up regulated lncRNA sirt1-as while it down regulated Aβ protein. As expected, the SIRT1 was significantly increased in SAMP8 mice after feeding by D-4F orally which implying that it may participate in the development of AD. This was partly in accordance with previous studies that SIRT1 play an essential role in AD (Bonda et al., 2011; Donmez, 2012) which encourage us to explore the precise mechanism underlying the sirt1-as/SIRT1 pathway.

In the previous researches, it has demonstrated that the SIRT1 can protect individuals from AD via the traditional mechanisms through BACE1 to reduce the production of Aβ protein (Marwarha et al., 2014). However, we did not observe any changes of BACE1 after treatment of D-4F in SAMP8 mice which was inconsistent with others' studies (Marwarha et al., 2014).

We further studied the SIRT1 downstream target proteins and discovered that the cholesterol efflux related proteins (LXRα and ABCA1) have been greatly up regulated by the higher dose of D-4F. These two protein play pivotal roles in the process of cholesterol efflux.

The previous studies have already indicated that cholesterol is of great importance in development of AD. Furthermore, the D-4F's contribution to the cholesterol efflux has also been identified. Thus, based on our results of D-4F on sirt1-as and the RCT related proteins, the D-4F may protect SAMP8 from AD via increasing sirt1-as and its downstream target proteins that play critical roles in RCT.

5. Conclusions

D-4F decreases the expression of Aβ protein through up-regulating long non coding RNA sirt1-as and its downstream proteins which may involve in reverse cholesterol transport, which warrants further mechanism studies.

Limitations

Our observational study has found the potential roles of D-4F in protection SAMP8 from AD. However, the precise mechanisms have not been identified in depth which warrants further basic studies. We will also investigate the underlying mechanisms via vitro experiments.

Authors' contributions

Xiao-Han Ding and Ping Ye designed this research. Xiao-Han Ding and Ping Ye also drafted the manuscript and performed the statistical analyses. Xiao-Han Ding, Jie Han, Yuan Liu and Ying Ji completed the animal experiments and data collection. Xiao-Han Ding and Jie Han have also performed the examinations of the related proteins by using qRT-PCT, Western Blot and immunohistochemistry experiments.

Conflict of Interest statement

There is no conflict of interests to declare.

Acknowledgments

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