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Studies on biological effect of lycopene on Hippocampus of hyperlipemia rats

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ABSTRACT

Objective: This study investigated into the effect of lycopene on expression of APP, bax and bcl-2 in hippocampal CA1 region of rats with hyperlipidemia. Methods: By total cholesterol (TC) and body weight, 48 adult male SD rats were randomized into six groups, a normal control group, fed with basic feed; a high-fat model group, fed with high-fat feed; a positive drug control group, fed with high-fat feed and administrated with fluvastatin sodium at a dose of 10 mg·kg·bw^{-1}·d^{-1} by gastric perfusion; and lycopene groups at three dose levels, fed with high-fat feed and administrated with lycopene at doses of 11, 22 and 44 mg·kg·bw^{-1}·d^{-1} respectively also by gastric perfusion. Caudal venous blood samples of rats in all groups were taken at week 0, week 1 and week 3 after the experiment started so as to assay TC, TG, LDL-C and HDL-C; at the end of the experiment, rat brains were taken and sections of the hippocampal CA1 region were prepared. Expression of APP, bax and bcl-2 in the CA1 region was determined by immunohistochemical methods and morphological examination was carried out. Results: One week after fed with high-fat feed, models of hyperlipidemia rats were established; at the end of experiment, hippocampal APP and bax expression was enhanced while bcl-2 expression was significantly weakened (p<0.05); to rats with hyperlipidemia, both lycopene and fluvastatin sodium could reduce TC, TG and LDL-C, inhibit expression of hippocampal APP and bax and promote expression of bcl-2 (p<0.05). Conclusion: Lycopene down-regulates the expression of bax and up-regulates that of bcl-2 mainly by reducing serum TC and LDL-C and weakening expression of APP in the hippocampal CA1 region of rats with hyperlipidemia, thereby maintaining normal morphology of hippocampal neurons and facilitating the protection of the brain.

Keywords: lycopene; hyperlipidemia; hippocampus; APP; bax; bcl-2

1. INTRODUCTION

Lycopene is a potent singlet oxygen scavenger and has the effect of preventing free radical injuries. Studies have shown that it has many important bioactivities, e.g., quenching singlet oxygen, eliminating reactive oxygen species, blocking lipid peroxidation, suppressing cell reproduction, reinforcing immunity, and inducing gap junction intercellular communication [1,2,3,4]. Both epidemiological and laboratorial studies suggest that lycopene, a powerful antioxidant agent, can reduce the risk of cardiovascular diseases [5,6]. Early studies indicate that lycopene can effectively lower levels of serum TC, TG and LDL-C of rabbits with artherosclerosis induced with high fat and inhibit atherogenesis with an effect equivalent to that of fluvastatin [7,8]. As is suggested by studies, hyperlipidemia can cause injuries to the brain. Rise of blood cholesterol may harm endothelial cells of cerebral arteries and capillaries, accelerate progress of atherosclerosis and slow cerebral blood flow, thereby injuring cerebral metabolism and increasing risks of cognitive function impairment and dementia. In addition, blood cholesterol increase may also directly lead to neuronal degeneration related to cognitive function. The biological mechanism [9] may be that blood cholesterol increase affects APP metabolism of neurons

and accelerates production and sediment of Aβ, thereby leading to cognitive function impairment. Every 10% increase of blood cholesterol can double the quantity of Aβ plaques in the brain [10]. Morphological changes of cranial neurons and decrease of cognitive ability both correlate with Aβ increase. Aβ can also mediate disorders of signal transduction pathways and lead to apoptosis in the end [11]. Lycopene can penetrate the blood-brain barrier and be present in the central nervous system [12]. Charu K. *et al* found that after eight weeks' feeding with lycopene at a dose of 10μg.kg.bw^{-1}, this substance became detectable at various concentrations from blood and tissue of mice and the lycopene concentration in the brain tissue was 9.24±3.19 ng.g^{-1} wet tissues, indicating that lycopene taken from food can pass through the blood-brain barrier and reach the brain tissue [13]. Studies carried out by Foy CJ *et al.* [14] showed that deficiency of a series of anti-oxidation nutrients including lycopene is related to such neurodegenerative diseases as Parkinson's disease, vascular dementia, Alzheimer disease, etc. To sum up, hyperlipemia can impair the brain tissue, while lycopene has blood lipid lowering action and can pass through the blood-brain barrier, so we think lycopene has protective effect on brains of hyperlipemia rats.

In this study, hyperlipidemia animal models were established by feeding rats with high-fat feed and then, lycopene was administrated to see its effect on serum lipid and expression of APP, bax and bcl-2 in the hippocampal CA1 region of the subject animals so as to investigate into the possible protective effect of lycopene on the hippocampus.

2. MATERIALS AND METHODS

2.1. Formulation and Supply of High-Fat Feed

Basic feed comprising wheat (20%), rice (20%), corn (10%), bean cake (24%), fish flour (10%), wheat bran (10%), salt (1%), bone meal (2%), milk powder (2) and multivitamins (1%) was prepared by Experimental Zoology Division, Xiangya Medical College of Central South University. The content of protein in the feed was 19% assayed by Kjeldah method.

High-fat feed: Animal models were established with reference to the method adopted by Deepa et al [15] using high-fat feed containing basic feed (94.5%), cholesterol (4%), cholic acid (1%) and propylthiouracil (0.5%).

2.2. Apparatus and Reagents

Amyloid precursor protein (APP) rabbit anti-mouse polyclonal antibodies (Lot: 20080601) were purchased from Wuhan Boster Bioengineering Co., Ltd; bax and bcl-2 rabbit anti-mouse polyclonal antibodies and DAB color development kits were all produced by Beijing Zhong Shan-Golden Bridge Biological Technology Co., Ltd and were expected to expire in July of 2009. Lycopene powder with a purity of 90% (Lot: 041202) was obtained from North China Pharmaceutical Co., Ltd. Fluvastatin sodium capsules (Lot: X0006) were supplied by Novartis AG. Kits of serum lipid indexes were produced by BioSino Bio-technology and Science Inc. Other instruments used in the study included a TP1020 auto dehydrating machine for organic tissue (Germany Leica), a ZT-14s staining machine (Yaguang, Xiaogan of Hubei), a Finesse325 common paraffin section machine (Thermo Shandon) and a BX40 light microscope (Olympus).

Fluvastatin is used together with lifestyle changes (diet, weight-loss, exercise) to reduce the amount of cholesterol (a fat-like substance) and certain other fatty substances in the blood. Fluvastatin is in a class of medications called HMG-CoA reductase inhibitors (statins). It works by slowing the production of cholesterol in the body. Fluvastatin sodium is an approved lipid lowering drug having passed numerous animal experiments and widely applied to the clinic. In animal experiments, the optimal dose for rats and rabbits is 10mg. kg-1 [16,17].

2.3. Animals and Grouping

48 adult male SD rats, weighing 195±10 g, were supplied by Experimental Zoology Division, Xiangya Medical College of Central University. After adaption feeding for one week with the basic feed, the animals were weighed on an empty stomach and caudal blood was taken to assay serum TC. Then, according to the body weight and TC level, the rats were divided randomly into six groups, each containing eight, and caged by two. Arranged were a normal control group (C), fed with basic feed; a high-fat model group (F), fed with high-fat feed; a positive drug control group (FF), fed with high-fat feed and administrated with fluvastatin sodium at a dose of 10 mg·kg·bw-1·d-1; and lycopene groups at three dose levels (FL1, FL2 and FL3), fed respectively with high-fat feed and administrated with lycopene at doses of 11, 22 and 44 mg·kg·bw-1·d-1. During the experiment, except group C, which was fed with basic feed, all groups were fed with high-fat feed. In the second week of the experiment, 1% CMC-Na solvent was administrated to group C and group F by gastric perfusion, and fluvastatin sodium and lycopene powder with 1% CMC-Na as the solvent were administrated, also by gastric perfusion, to animals in other groups at the designed doses (the gastric administration volume was 1 ml·d-1 for rats in all groups). The animals were allowed to drink freely and their daily food intakes were recorded. The temperature and relative humidity were respectively controlled at 25±2℃ and 60%~70%. The rats were weighed twice a week and the gastric administration volumes of fluvastatin sodium and lycopene

were adjusted according to the body weights. However, since the dose relative to body weight remained constant, gastric administration was performed according strictly to the designed doses. All experimental procedures were conducted in accordance with the guidelines of the animal ethical committee for animal experimentation in China.

3. METHODS

3.1. Specimen Collection

At the ends of week 0, week 1 and week 3 of the experiment, the animals were fasted for 12h and then, their tails were cut; blood samples were taken to assay the levels of serum TC, TG, LDL-C and HDL-C. At the end of the experiment, pentobarbital sodium at a dose of 40mg.kg. bw-1 was administrated by peritoneal injection to anesthetize the animals, and then, the animals were deeply anesthetized and perfused via left ventricular with 0.9% saline followed by 4% paraformaldehyde (20ml/min for 5 min). Following decapitation, the brains were removed. After the cerebral meninges was stripped, the brains was fixed in 10% formaldehyde solution .And 24h later, the specimen was cut with a coronal-shaped opening about 5mm in front of the posterior extremity of the biparietal suture; from the plane of the hippocampus and the dentate band under macroscopic observation, a tissue piece about 1cm thick was cut in the direction of the procerebrum and imbedded with paraffin. The coronal plane was cut continuously to produce eight sections with a thickness of 4μm for each rat. According to the preparation order, sections of each group were divided into eight sets. Immunohistochemical staining was applied to sections of the 1st, 2nd, 4th, 5th, 6th and 8th sets, while HE routine staining was applied to the 3rd and 7th sets.

3.2. Assay Methods

Serum TG and TC were analyzed by enzymatic methods of glycerol phosphate oxidase-peroxidase- 4- aminoantipyrine (GPOPAP) and cholesterol oxidase-peroxidase-4-aminoantipyrine (CHOD-PAP) respectively) [18]. Concentrations of HDL-C were determined in the supernatant after precipitation of lipoprotein-B using phosphototungstic acid/$Mg2^{+}$ (PTA/$Mg2^{+}$), and the concentrations of LDL-C were calculated as described by Friedewald *et al*. [19]. All the operation procedures were carried out according to instructions of the kits. Immunohistochemical SABC [20] or HE staining of the histological sections was performed to detect APP, bax and bcl-2 positive particles and pathological changes of the hippocampal CA1 region. The principal procedures included paraffin section deparaffinage and dehydration; 3% H_2O_2 incubation for 20min; phosphate buffer (PBS) washing; microwave treatment in 0.01mol/L citrate buffer for antigen retrieval; blocking serum application and incubation for 20min; primary antibody application and incubation at 37℃ for 1-2h; horse radish peroxidase labeled secondary application and incubation for 15min; color development with diaminobenzidine (DAB) solution; hematine counterstaining; dehydration; mounting; and light microscopic observation. For the negative control, the operation was basically the same as that for the test groups except that the primary antibody was substituted by phosphate buffer. Presence of brown-yellow particles in the nucleus or the endochylema was considered as indication of positive results. Under a 400× high power lens, 10 fields of view without edge overlap were selected randomly for each section (five fields of view for the hippocampal CA1 region of each side, each field being about 0.075 squm) to count positive particles in each high power field. The number of positive particles was calculated by the following formula: number of positive particles = (the total number of positive cells/the total number of cells)/10 (the number of fields of view). The result was expressed as the mean [21]. Pictures were taken.

3.3. Quality Control

All glassware used for the experiment were soaked in a sulfuric acid solution of potassium bichromate for 24h, washed clean with deionized water, rinsed with redistilled water for three times and then dried for use. A certain quantity of paraffin sections was drawn for preliminary experiment of immunohistochemical staining so as to elicit the optimal experimental conditions. Lycopene was dissolved in 1% CMC-Na and the gastric administration solution was freshly prepared before use.

3.4. Statistical Methods

The data were analyzed with SPSS13.0 software and all measurement data were expressed as mean ± standard deviation ($\bar{x}$ ±S). Analysis of variance was used for group comparisons. Statistical analyses were performed using analysis of variance for mean comparison of multiple samples, S-N-K and LSD tests for paired comparison, and analysis of variance of data of replicate measurements for comparison of different experiment intervals. Kruskal-Wallis H test and Spearman correlation analysis were applied to data in non-normal distribution, which were also tested by Nemenyi method of paired comparison. The level of statistical significance for all analyses was set at α=0.05.

4. RESULTS

4.1. General Conditions and Body Weight Changes of Rats

During the experiment, rats of all groups exhibited normal activities and had smooth and glossy fur as always.

No significant difference was found in body weight in all groups with the exception of group C at all time points during the experiment (P>0.05). Analysis of variance of replicate measurements showed that, in different stages, body weights of the animals in all groups have significant difference (P<0.05).

4.2. Serum Lipid Index Changes of Rats in Different Stages

Analysis results of serum lipid indexes of rats in different stages are shown in **Figure 1**.

As is indicated by the analysis results in **Figure 1**, at the end of the first week of the experiment, serum TC, TG and LDL-C of rats in the group fed with high-fat feed increased significantly (P<0.05) compared with those of rats in group C, suggesting the success of establishment of hyperlipidemia rat models with no statistical significance of lipid index differences among the groups (P>0.05). At the end experiment, no significant difference was present between TC levels of group FL3 and group FF (P>0.05), neither between TG levels of group FL2 and FL3 and group FF (P>0.05). Compared with the result of group F, the LDL-C levels of all other groups were significantly decreased (P<0.05); the LDL-C level of group FL3 was lower than that of group FF (P<0.05). HDL-C differences of rats in these groups during the experiment had no statistical significance (P>0.05).

4.3. Expression of APP in the Hippocampal CA1 Region

Positive APP reaction products are brown-yellow particles distributed in cell membranes and cytoplasma. The analysis results in **Table 1** show that: the number of positive APP particles in group F increased significantly compared with that in group C (P<0.05); the number of APP positive particles in groups FF, FL2 and FL3 was significantly lower than that in group F (P<0.05); the difference between group C and group FL1 had no statistical significance (P=0.492); the difference of the

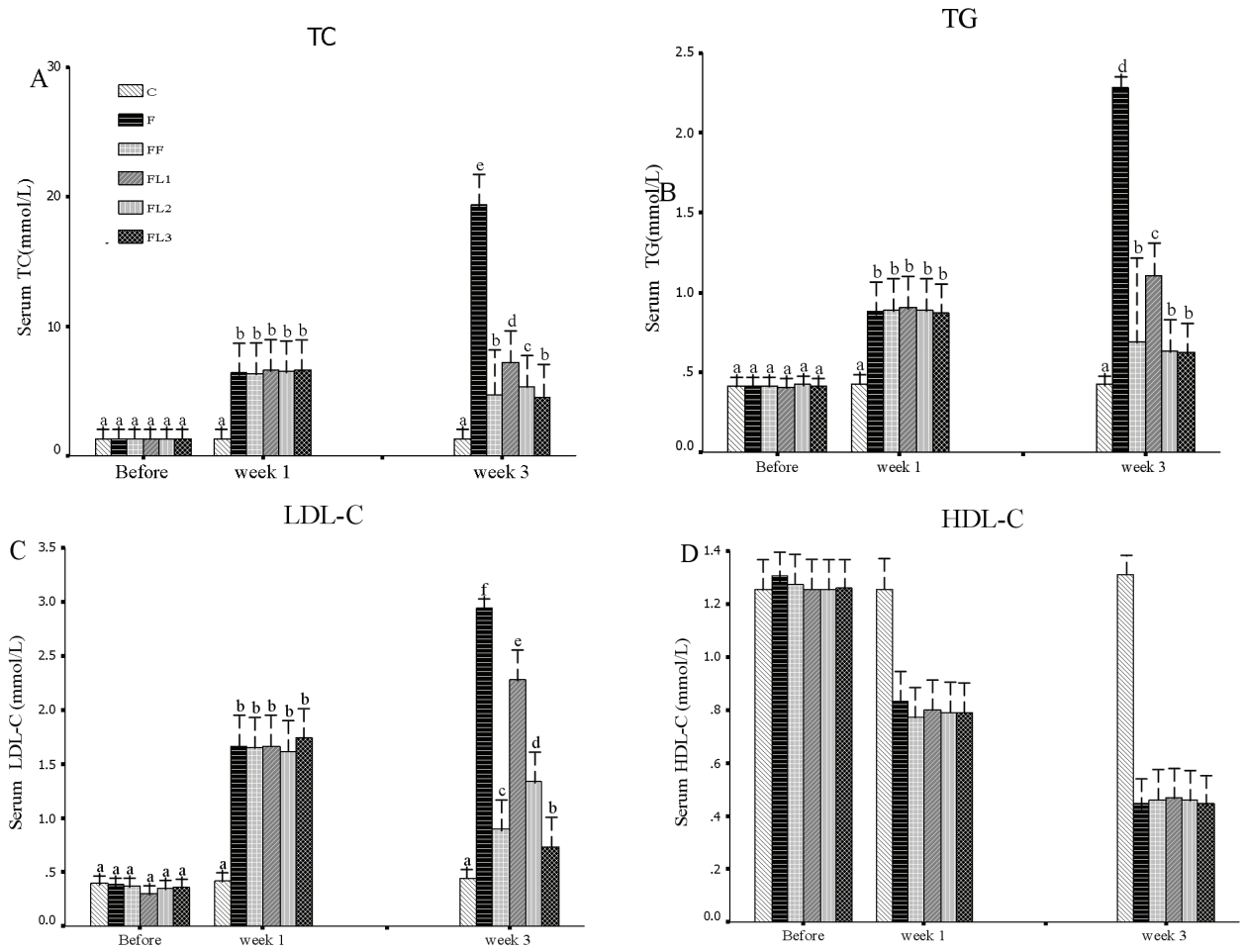

Figure 1. The serum lipids of rats at different experimental stage ($\bar{x}\pm S$). Different alphabet superscripts of the same column indicate the significant differences between groups (p<0.05); Different symbol (*, #, &) represent significant differences at 0, 1, 3w in the same group (p<0.05).

number of APP positive particles in group FL2 and group FL3 had no statistical significance (P=0.535). A few APP positive reaction particles could be seen in the hippocampal CA1 region of rats in group C. The quantity of positive particles significantly increased and the color was rather deep in group F. The above findings indicated that lycopene can inhibit expression of APP in hippocampal CA1 region of rats with hyperlipidemia. The effect in lycopene groups of 22mg·kg·bw^{-1}·d^{-1} and 44mg·kg·bw^{-1}·d^{-1} was more satisfactory than that in the 10mg·kg·bw^{-1}·d^{-1} fluvastatin sodium group (see **Figure 2**).

4.4. Expression of bax and bcl-2 in the Hippocampal CA1 Region

After immunohistochemical staining of neurons, the main phenomenon was the presence of brown-yellow substance depositing in cytoplasm. Part cell membrane and caryotheca were also stained with the manifestation of neuron profiles. According to result analysis in Table 2, bax and bcl-2 were both expressed in hippocampal CA1 region of rats in group C; in rats of group F, the number of bax positive particles significantly increased while that of bcl-1 positive particles significantly decreased (P<0.05); the number of bax positive particles in groups F, FL1 and FL2 was higher than in group FF and the differences were statistically significant (P<0.05). the number of bax positive particles in groups FF, FL3 with differences of no statistical significance (P=0.957); the number of bcl-2 positive particles in groups FF, FL1, FL2 and FL3 were higher than in group FF with differences of statistical significance (P<0.05). As is shown in **Figure 3**, bax-expression neurons degenerated with cell shape irregularity and the cell edges were not so smooth. **Figure 4** indicates that bcl-2-expression neurons had nearly normal shapes; the cells didn't swell and were mostly oval-shaped, and the edges were smooth.

4.5. Pathological Changes of Hippocampal CA1 Region

By HE staining, in observations under high power lenses,

Table 1. The expression APP in the hippocampus CA1 region [Median(QR)]. Different alphabet superscripts of the iso-column indicate the significant differences between groups (a: p<0.05,vs. group C; b: p<0.05, vs. group F; c: p<0.05, vs. group FF).

Group	N	App
C	8	26.5 (4.5) $^{b c}$
F	8	58.5 (10.3) $^{a c}$
FF	8	2.0 (3.3) $^{a b}$
FL1	8	25.0 (3.5) $^{b c}$
FL2	8	0.0 (0.0) $^{a b}$
FL3	8	0.0 (0.8) $^{a b}$

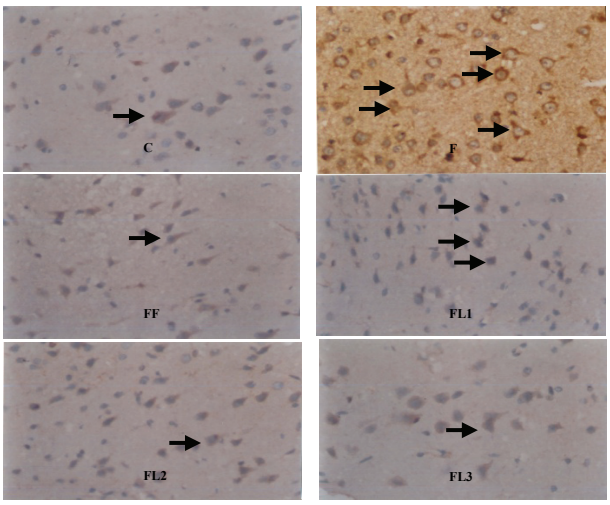

Figure 2. The hippocampus APP change of rat with immunohistochemistry by using the high power light microscope (×400). Panel C: control; Panel F: hyperlipidemia model; Panel FF: positive control, fluvastatin sodium; Panel FL1: 11mg·kg·bw^{-1}·d^{-1} of lycopene; Panel FL2: 22mg·kg·bw^{-1}·d^{-1} of lycopene; Panel FL3: 44mg·kg·bw^{-1}·d^{-1} of lycopene.

Table 2. The expression bax and bcl-2 in the hippocampus CA1 region [Median(QR)]. Different alphabet superscripts of the iso-column indicate the significant differences between groups (a:p<0.05, vs. group C; b:p<0.05, vs. group F; c: p< 0.05, vs. group FF).

Group	N	bax	bcl-2	bcl-2/bax
C	8	6.5(1.8) $^{b c}$	15.5(2.8) $^{b c}$	2.31 $^{b c}$
F	8	60.5(11.0) $^{a c}$	0.0(0.8) $^{a c}$	0.00 $^{a c}$
FF	8	11.0(3.0) $^{a b}$	16.0(2.5) ^{b}	1.53 $^{a b}$
FL1	8	26.0(4.5) $^{a b c}$	23.5(4.8) $^{a b c}$	0.90a $^{b c}$
FL2	8	22.5(3.3) $^{a b c}$	49.0(8.8) $^{a b c}$	2.18 $^{b c}$
FL3	8	11.0(2.0) $^{a b}$	67.5(4.8) $^{a b c}$	6.06 $^{a b c}$

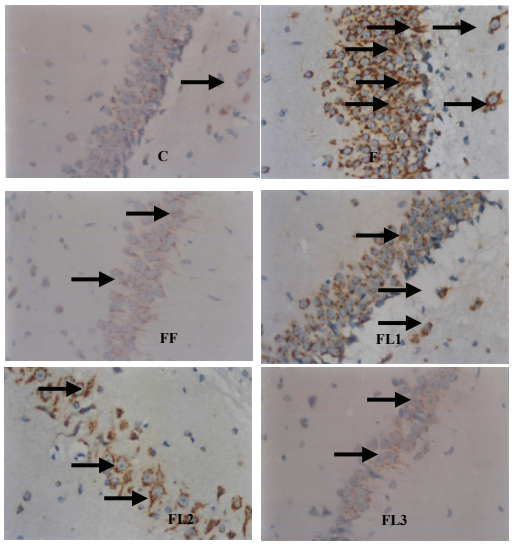

Figure 3. The hippocampus bax change of rat with immunohistochemistry by using the high power light (×400). Panel C:control; Panel F; hyperlipidemia model; Panel FF; positive control, fluvastatin sodium; Panel FL1: 11mg·kg·bw^{-1}·d^{-1} of lycopene; Panel FL2: 22mg·kg·bw^{-1}·d^{-1} of lycopene; Panel FL3: 44mg·kg·bw^{-1}·d^{-1} of lycopene.

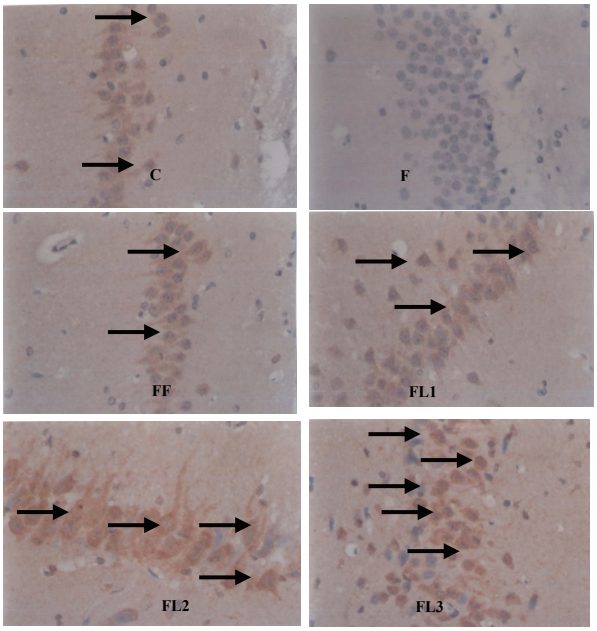

Figure 4. The hippocampus bcl-2 change of rat with immunohistochemistry by using the high power light (×400). Panel C: control; Panel F: hyperlipidemia model; Panel FF: positive control, fluvastatin sodium; Panel FL1: 11mg·kg·bw^{-1}·d^{-1} of lycopene; Panel FL2: 22mg·kg·bw^{-1}·d^{-1} of lycopene; Panel FL3: 44mg·kg·bw^{-1}·d^{-1} of lycopene.

the rats in group F of the number of hippocampal pyramid cells decreased; the neurons were disorderedly arranged and were mostly stained deeply and triangular with degeneration and karyopyknosis. In contrast, the rats in other groups, large amounts of hippocampal pyramid cells arranged tightly and regularly , clear profiles and distinct demarcation between surrounding tissue; the nuclei were blue and the cytoplasm was light red; in shape, size and arrangement, neurons of all groups were approximately normal (see **Figure 5**).

4.6. Correlation between the Parameters

Correlation analyses of TC, LDL-C and TG data in **Figure 1** and APP data in Table 1 found that Spearman's rho was 0.821, 0.785 and 0.695 respectively and P values were all 0.000, so it can be considered that TC and LDL-C changes are closely related to excessive expression of APP. Findings in this study showed that hyperlipidemia could lead to excessive expression of APP and bax in hippocampal CA1 region of rats and their expressions were positively correlated, where Spearman's rho was 0.774 and *P* was 0.000; bcl-2 expression was reduced and was in negative correlation with APP expression, where Spearman's rho was −0.737 and *P* values was 0.000; bcl-2/bax ratio decreased and was significantly lower than that of the normal control group ($P<0.05$).

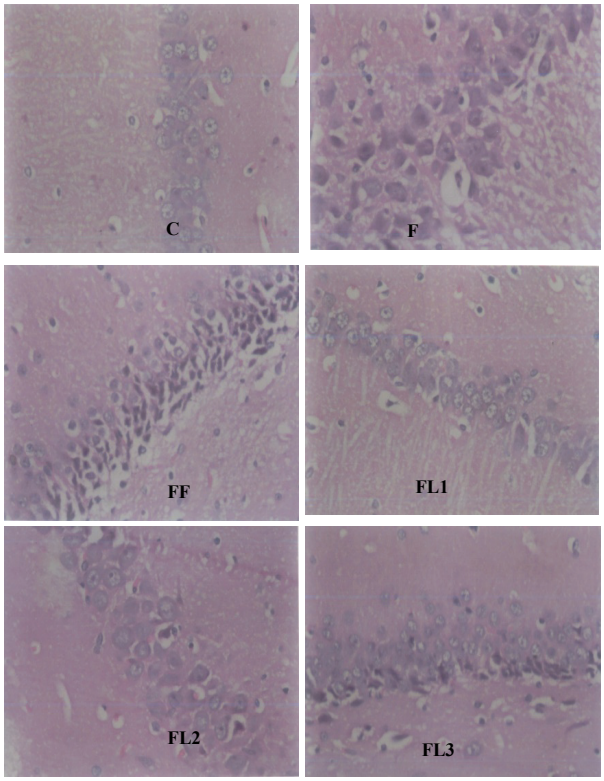

Figure 5. The hippocampus change of rat dyed with HE by using the high power light (×400). Panel C: control; Panel F: hyperlipidemia model; Panel FF; positive control, fluvastatin sodium; Panel FL1: 11mg·kg·bw^{-1}·d^{-1} of lycopene; Panel FL2: 22mg·kg·bw^{-1}·d^{-1} of lycopene; Panel FL3: 44mg·kg·bw^{-1}·d^{-1} of lycopene.

5. DISCUSSION

Hyperlipidemia is closely related to the occurrence of coronary heart diseases, myocardial infarction, hyper tension, diabetes mellitus, apoplexy, etc. and also poses a risk of AD. Results of this study also suggest that high-fat feed intake can cause hyperlipidemia in rats while lycopene intervention can lower the serum lipid level of rats fed continually with high-fat feed and decelerate the rise of serum lipid.

Clinical studies have also confirmed the positive correlation between excessive increase of serum cholesterol and AD occurrence [22]. In recent years, some studies showed that hyperlipemia is an important factor for development and progress of AD [23,24]. Yanagisawa [25] et al found that the level of low density lipoprotein cholesterol in the serum of AD patients significantly increased, while that of high density lipoprotein cholesterol significantly decreased. Epidemiological studies proved that old people with blood cholesterol are more subject to AD [26,27] and that people with hypercholesteremia in middle age are more prone to suffer from AD when old [28]. Cholesterol lowering measures, especially taking statins, can reduce the risk of AD [29,30]

and relieve cognitive function impairment of AD patients [31]. A retrospective study on a population taking statins showed that the AD incidence rate was 70% lower in people taking the drugs than in the control group [32]. It is a characteristic pathological change of AD that β-amyloid (Aβ) deposits abnormally in special encephalic regions[33,34] and the development of Aβ is intimately connected to cholesterol level [35,36]. Basic studies have proved that high cholesterol is related to cognitive function impairment and that intake of foods causing atherosclerosis can aggravate depletion of learning abilities of APP transgenic mice [37]. In vitro cell culture experiments suggested that whether by inhibiting cholesterol synthesis with HMG-CoAR inhibiters or by lowering intracellular cholesterol with physical methods, cellular Aβ content could be reduced [38].

APP is a transmembrane glycoprotein, mainly in the central nervous system. The normal physiological function (s) of APP in learning and memory remains unclear. APP physiologically processed by site-specific proteolysis firstly by α- secretases or β-secretases, releasing a large fragment called APP (S) that contains most of the extracellular sequences of APP, a small extracellular stub, the transmembrane region and the cytoplasmic tail of APP. These are subsequently cleaved by γ-secretase at multiple sites in the transmembrane region, releasing small peptides, Aβ (1-40) and Aβ (1-42), the major components of AD-associated amyloid fibrils [39]. Aβ, is a principal constituent of senile plaques (degenerated axons surrounding amyloid substance in essence, one of the main pathological changes of AD) [40]. Then the cholesterol level in the blood rises, more Aβ will be produced in the brain [41]. Morphological changes of cranial neurons and decrease of cognitive ability both correlate with Aβ increase. Aβ can also mediate disorders of signal transduction pathways and lead to apoptosis in the end [42]. It has been found in studies that excessive expression of APP genes is the cause for deposition of β-amyloid [43] and that the contents of β-APP mRNA and APP protein in hippocampal region of senescence accelerated mice (SAM) increase with the growth of age and excessive expression of hippocampal APP is related to memory loss of SAMs [44]. This study suggests content increase of serum TC, LDL-C and TG and also excessive APP expression in hippocampal CA1 region of hyperlipidemia rat models established after feeding high-fat feed for three weeks. Lycopene can inhibit excessive APP expression by lowering serum TC and LDL-C levels of rats, reducing injuries to the nervous system caused by hyperlipidemia. Analysis results in Table 1 indicate that APP expression of fluvastatin sodium group and lycopene group of 22 and 44 $mg \cdot kg \cdot bw^{-1} \cdot d^{-1}$ was significantly lower than that of the normal control group. So far, there has been no study report on low expression of APP. In this study, no marked abnormality was found in hippocampus morphology observation of rats in the above three groups. Whether lycopene plays its hippocampus protection role through other routes and whether long-term low APP expression will bring adverse effect on the organic body should be addressed in further studies.

Neuron apoptosis is necessary to dynamic equilibrium maintenance of growth and development of the nervous system and takes place mainly in early stages of the nervous system development, when more than half of maturing neurons will be cleared away; apoptosis can also remove injured or diseased neurons [45]. However, apoptosis is also an important way of neuron dying; excessive apoptosis may aggravate cerebral ischemic-reperfusion injuries [46]. Apoptosis is the cause for cognitive impairment before massive neuron death [47]. It has been proved in studies that neuron loss in AD originates from apoptosis, evidenced by that the number of TUNEL positive cells is found by TUNEL method to increase in AD autopsied brain tissue [48] and that apoptosis-related bcl-2 is found be down-regulated in brains of AD patients [49,50]. Genes related to cerebral neuron apoptosis include apoptosis inhibiting genes and apoptosis inducing genes. Bcl-2 is an apoptosis inhibiting gene while genes inducing apoptosis mainly include bax, Fas, p53, etc. Bcl-2 and bax are in a balanced system in healthy people. Excessive expression of bcl-2 will inhibit apoptosis while excessive expression of bax can accelerate apoptosis. The bcl-2/bax ratio regulates the occurrence of apoptosis. A study showed by maintaining the local ratio of bcl-2/bax using the HSV bcl-2 vector one may protect CA1 pyramidal cell from the delayed neuronal death of transient global ischemia [51].

As is suggested in in vivo and in vitro studies, APP can down-regulate expression of bcl-2 while up-regulate that of bax [52]. Morphological observation showed that hippocampal pyramidal cells of the model group decreased and were arranged disorderedly while morphology of hippocampal region of other groups was basically normal. The study indicates that lycopene can maintain morphological normality of hippocampal neurons of hyperlipidemia rats and, possibly by inhibiting excessive expression of hippocampal APP, up-regulate expression of apoptosis inhibiting gene bcl-2, down-regulate expression of apoptosis promoting gene bax and maintaining constancy of bcl-2/bax ratio so as to protect hippocampal neurons.

To conclude, lycopene can effectively reduce serum lipid level of experimental hyperlipidemia rats with the dose of $44mg \cdot kg \cdot bw^{-1}$ having the most marked effect. Lycopene down-regulates the expression of bax and up-regulates that bcl-2of mainly by reducing serum TC and LDL-C and weakening expression of APP in the hippocampal CA1 region of rats with hyperlipidemia, thereby facilitating the protection of the brain. However, the specific working mechanism, the optimal effective doses in different tissues and the adverse effect still re-

quire further studies at both cellular and overall levels.

Abbreviations

LDL: lowdensity lipoprotein; HDL:high density lipoprotein; TC: total cholesterol; TG: triglycerides; PBS: phosphate buffer saline; DAB: diaminobenzidine; AD: Alzheimer disease; APP: amyloid protein precursor; Aβ: β-amyloid

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

YZ carried out the experiment of this manuscript and drafted the manuscript and approved the final manuscript; MH and SQ participated in the design of the study and revised the manuscript. LZ participated the experiment.

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