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Effect of β-casomorphin-7 on myocardial hypertrophy in hyperthyroidism-induced cardiomyopathy

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Abstract. – OBJECTIVE: The purpose of this study was to investigate the effect of β-casomorphin-7 (β-CM-7) on myocardial hypertrophy (MH) in hyperthyroidism-induced cardiomyopathy *in vivo* and *in vitro*.

MATERIALS AND METHODS: Thirty C56BL/6 mice were randomly divided into three groups: control group, hyperthyroidism group, and β-CM-7 treatment group. An animal model of cardiac hypertrophy of hyperthyroid heart disease (HHD) was constructed by continuous intraperitoneal injection of 100 μg of L-thyroxine (L-Thy) for 28 days, and the serum TT3 and TT4 concentrations were measured. After that, myocardial specimens were collected to measure left and right ventricular MH index, and the myocardial cell structure was observed under hematoxylin and eosin (HE) staining. Thereafter, Masson staining was adopted to determine collagen volume fraction, and hydroxylamine method was used to measure superoxide dismutase (SOD) activity, Meanwhile, DTNB direct method was applied to measure GSH-Px activity, thio-malonylurea method was utilized to measure malondialdehyde (MDA) content, and the level of reactive oxygen species (ROS) was detected by flow cytometry. Finally, the expressions of oxidative stress (OS) and inflammation-related factors *in vivo* and the nuclear factor-κB (NF-κB) pathway *in vitro* were detected by Western blot and quantitative real-time polymerase chain reaction (qRT-PCR).

RESULTS: Compared with those in control group, TT3 and TT4 were remarkably increased, the structure of myocardial cells was disordered, the interstitial fibrosis and the ventricular MH index were significantly increased, the OS and inflammatory responses were increased, and the NF-κB pathway was activated in the Hyperthyroidism group. In the β-CM-7 group, the content of TT3 and TT4 was decreased, the myocardial cell structure was slightly disturbed, the fibrosis and the ventricular MH index were reduced, OS and inflammatory response were reduced, and the NF-κB pathway was inhibited.

CONCLUSIONS: β-CM-7 can prevent and treat MH in mice with L-Thy-induced HHD probably through regulating the NF-κB signaling pathway.

Key Words:
β-casomorphin-7, Hyperthyroidism myocardial, Hypertrophy, NF-κB signaling pathway.

Introduction

Hyperthyroidism refers to the persistently high-functioning state of the thyroid gland, which synthesizes and releases too much thyroxine, thus resulting in clinical symptoms such as thyrotoxicosis[1]. The heart is one of the target organs of thyroxine. Excessive thyroid hormone can cause heart damage, arrhythmia, cardiac hypertrophy and heart failure[2]. Heart disease triggered by hyperthyroidism is called hyperthyroid heart disease (HHD), which refers to a series of cardiovascular diseases such as cardiac enlargement, cardiac insufficiency, and atrial fibrillation caused by direct or indirect effects of excessive thyroid hormones on the heart during hyperthyroidism. Systemic symptoms and signs of an endocrine disorder of heart disease can occur in any patient with hyperthyroidism, and its prevalence is about 10%-22% of patients with hyperthyroidism[3,4]. HHD, a serious complication of hyperthyroidism, is one of the leading causes of death from hyperthyroidism. Myocardial hypertrophy (MH) is an independent risk factor for cardiovascular accidents. Patients with MH

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have a remarkably increased risk of arrhythmia and sudden death, so it is more important than other risk factors, such as smoking and hypercholesterolemia. Cardiovascular incidence rates in MH patients are 2 to 4 times higher than that in patients without MH[5]. Therefore, the immediate and effective prevention of MH is a reliable way to reduce the mortality and morbidity of cardiovascular disease.

β-CM-7 is a heptapeptide with opioid activity isolated from the enzymatic hydrolysis of cow's milk β-casein. It is a 60-66 amino acid residue fragment of β-casein, and has a good affinity with u-type receptors[6]. β-CM-7 is casein released by protease hydrolysis under certain conditions and then produces antioxidant effects *in vivo*[7].

Nuclear factor-κB (NF-κB) is found in a variety of cells, such as vascular endothelial cells, vascular smooth muscle cells, and cardiomyocytes, and is involved in gene regulation of various physiological and pathological processes such as inflammation, oxidative stress (OS), cell proliferation, and apoptosis[8]. Normally, NF-κB combines with its endogenous inhibitory protein (I-κB) to form a trimer in the form of homo- or heterodimer. It exists in the cytoplasm in an inactive state. Its most active form is a p50/p65 heterodimer. When I-kB is phosphorylated and dissociated from the NF-κB/I-κB complex, the dimer and inhibitor protein dissociate, and the dimer then transfers to the nucleus, where it binds to the kB site on DNA and participates in the transcription of multiple genes[9]. Raish et al[10] have shown that by regulating the NF-κB signaling pathway, OS and inflammatory responses can be suppressed. Thus, it was speculated that β-CM-7 can also regulate the NF-κB pathway, thereby inhibiting OS and inflammatory responses induced by HHD.

Materials and Methods

Experimental Animal

A total of 30 male C56BL/6 mice (Shanxian Central Hospital Animal Center) aged 8 weeks old, weighing (20 ± 2) g were included in the experiment. After 1 week of adaptive feeding, no adverse reactions occurred, and the mice underwent normal diet and drinking, with normal body weight, temperature and activity. This investigation was approved by the Animal Ethics Committee of Shanxian Central Hospital Animal Center.

Preparation of Hyperthyroid Cardiomyopathy Model

0.1 g/L L-Thy (Tianpu Biochemical Pharmaceutical, Guangzhou, China) was mixed with 5 g/L sodium carboxymethylcellulose (Tianpu Biochemical Pharmaceutical, Guangzhou, China) and thoroughly ground before use. Mice were injected intraperitoneally with 100 μg/kg L-Thy for 28 days to establish a mouse cardiac hypertrophy model.

Experimental Grouping

Thirty C56BL/6 mice were randomly numbered and divided into three groups. The control group was intraperitoneally injected with 5 g/L sodium carboxymethylcellulose daily for 28 days. In hyperthyroidism group and β-CM-7 group, mice were injected intraperitoneally with 100 μg/kg L-Thy for 28 days. In β-CM-7 group, after the mouse model of MH was established, 15 μmol/L β-CM-7 (Tianpu Biochemical Pharmaceutical, Guangzhou, China) was continuously subjected to gavage for 30 days. In addition, the control group and the hyperthyroidism group were given the same amount of normal saline daily.

Cell Culture and Processing

H9c2 cells (Cell Culture Center, Shanghai, China) were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Life Technology, Wuhan, China) containing 10% fetal bovine serum (FBS) (Life Technology, Wuhan, China) and 1% penicillin/streptomycin (Life Technology, Wuhan, China). When the cell density reached about 80%, the cells were digested with trypsin (Life Technology, Wuhan, China) and passaged at intervals of 1-2 days. H9c2 cells in logarithmic growth phase were plated in 6-well plates at $5'10^4$ per well. The control group was cultured in normal medium, and the model group was treated with 2 μM of L-Thy for 24 hours. In β-CM-7 group, H9c2 cells were previously cultured with 10^{-6} mol/L β-CM-7 for 6 h, and then co-cultured with L-Thy-containing medium for 24 h.

Cell Counting Kit-8 (CCK-8) Assay

H9c2 cells were inoculated in a 96-well plate with 2000 cells per well, and then treated with β-CM-7 at different concentrations the next day. Then, the CCK-8 reagent (Jian Cheng, Nanjing, China) was added in the dark for incubation at 37°C for 2 h, and the absorbance was detected at 450 nm.

Tissue Sampling

After 30 days, mice in each group were weighed and anesthetized. Then, the blood was collected from the jugular vein and centrifuged to obtain serum. Thereafter, the mice were executed to quickly remove the heart after cardiac perfusion. The next day, a clean absorbent paper was used to absorb the water on the heart surface. Finally, some heart tissues were taken using paraformaldehyde, and the remaining tissues were stored at -80°C.

Ventricular Hypertrophy Index Calculation

Left ventricular wet weight/body weight (LVW/BW, mg/g) and right ventricular wet weight/body weight (RVW/BW, mg/g) were calculated, representing left ventricular MH index and right ventricular MH index.

Hematoxylin-Eosin (HE) Staining

The mouse heart tissues were fixed with formaldehyde, cut into 3 μm-thick sections (German LEICA, Wetzlar, Germany), stained according to the kit's instructions (Jian Cheng, Nanjing, China), sealed with a sealing liquid and finally observed with an optical microscope.

Masson Stain

Masson staining was adopted for observing the degree of cardiac fibrosis. After staining according to the kit instructions (Jian Cheng, Nanjing, China), the slides were sealed with a sealing liquid and observed under a light microscope. Thereafter, 10 different fields of view of each slice were observed under a high-power microscope, the ratio of collagen fiber green stained area to field area was calculated, and the results were averaged.

Immunofluorescence

H9c2 cells in the logarithmic phase were cultured in 12-well plates at $2'10^4$ per well. After drug treatment, the cells were fixed with 4% paraformaldehyde, and treated with Triton X-100 (Thermo Fisher Scientific, Waltham, MA, USA) for 15 min. Subsequently, the cells were blocked by 10 % goat serum for 1 h, and incubated at 4°C overnight with P-p65 (Abcam, Cambridge, MA, USA, Rabbit, 1:1000). The next day, they were washed 3 times with phosphate-buffered saline (PBS), incubated with secondary antibody for 1 h in the dark, and then added 4',6-diamidino-2-phenylindole (DAPI) in the dark. Finally, these cells were collected and observed under a fluorescence microscope.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The heart tissues and H9c2 cells in each group were tested. RNA was extracted from tissues and H9c2 cells by TRIzol using an extraction kit (Thermo Fisher Scientific, Waltham, MA, USA) and then reversely transcribed into complementary deoxyribose nucleic acid cDNA. After that, the transcribed cDNA was analyzed by real-time PCR. Primer 5.0 software was used to design primers, which were shown in Table I.

Western Blot

The heart tissues and H9c2 cells in each group were added with radioimmunoprecipitation assay (RIPA) lysate (Camilo Biological, Nanjing, China) and homogenized on ice. After centrifugation, the supernatant was taken and denatured by protein loading buffer. After electrophoresed, the protein was transferred to a polyvinylidene difluoride (PVDF, Millipore,

Table I. Real time PCR primers.

Gene name	Forward (5′>3′)	Reverse (5′>3′)
SOD1	GGTGAACCAGTTGTGTTGTC	CCGTCCTTTCCAGCAGTC
SOD2	CAGACCTGCCTTACGACTATGG	CTCGGTGGCGTTGAGATTGTT
IL-1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
TNF-α	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
IκBα	GGATCTAGCAGCTACGTACG	TTAGGACCTGACGTAACACG
P65	ACTGCCGGGATGGCTACTAT	TCTGGATTCGCTGGCTAATGG
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

RT-PCR, quantitative reverse-transcription polymerase chain reaction.

Billerica, MA, USA) membrane, then blocked with skim milk powder, and incubated with specific antibodies [SOD1, Abcam, Cambridge, MA, USA, 1:2000, SOD2, Abcam, Cambridge, MA, USA, 1:2000, IL-1β, Abcam, Cambridge, MA, USA, 1:2000, TNF-α, Abcam, Cambridge, MA, USA, 1:1000, p65, Abcam, Cambridge, MA, USA, 1:1000, P-p65, Abcam, Cambridge, MA, USA, 1:2000, IκBα, Abcam, Cambridge, MA, USA, 1:2000, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), Abcam, Cambridge, MA, USA, 1:2000] overnight. Next day, the corresponding secondary antibody (Yifei Xue, Nanjing, China, 1:3000) was used for continuous incubation, and the images were collected and processed after exposure to enhanced chemiluminescence (ECL), and GAPDH was used as an internal reference. The results were expressed as the ratio of the absorbance of the target band to GAPDH.

Determination of Serum TT_3, TT_4 Levels

TT_3 and TT_4 were determined by solid-phase microsphere method according to the kit instructions (Jian Cheng, Nanjing, China).

Biochemical Indicator Detection

As per the instructions of the SOD, GSH-Px and MDA kits (Jian Cheng, Nanjing, China), appropriate amounts of serum were taken and the corresponding index data were tested.

Flow Cytometry to Detect ROS Levels

After the mice were anesthetized, the heart tissues were quickly removed, placed in 1 mL of ice-PBS solution, and cut into pieces. First, the cells were filtered with a sieve and centrifuged for 5 min, and the supernatant was discarded. Then, a cold PBS solution was added to prepare a single cell suspension, and 5 μL of 2', 7'-dichloro fluorescent yellow diacetate (DCF-DA, Kaiji, Nanjing, China) was added, followed by cell incubation in a 37°C incubator for 30 min in the dark. Following centrifugation, the supernatant was discarded, 10% FBS was added, and the cells were incubated at 37°C for 20 min. After centrifugation, the supernatant was discarded, and an appropriate amount of cold PBS solution was added to prepare a single cell suspension of heart tissues. Finally, a flow cytometer was utilized to measure the average fluorescence intensity of the labeled fluorescent probe in the cells, and the fluorescence intensity value was recorded.

Enzyme-Linked Immunosorbent Assay (ELISA)

The tissues were taken from each group of mice, and an appropriate amount of PBS was added to the heart tissues, the homogenate was centrifuged for 10 min, and the supernatant was collected. Next, the content of TNF-α and IL-1β in serum was detected by ELISA, in strict accordance with the instructions of ELISA kit (Elabscience, Wuhan, China).

Statistical Analysis

All data were analyzed using Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) and expressed as mean ± SD (standard deviation). Differences between two groups were analyzed using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by post-hoc test (Least Significant Difference). $p<0.05$ represented that the difference was statistically significant.

Results

β-CM-7 Relieved L-Thy-Induced Cardiac Structural Changes

First, to verify the success of the modeling, the serum levels of TT_3 and TT_4 were first tested in the hyperthyroidism group, and it was found that the levels of TT_3 and TT_4 in the hyperthyroidism group were significantly higher than those in the control group (Table II). At the same time, analysis of the left and right ventricular MH indexes also confirmed that the left and right ventricular MH indexes in the hyperthyroidism group were dramatically higher than those in the control group (Table III). Therefore, the mouse cardiac hypertrophy model was successfully prepared. In addition, it was discovered that after treatment with β-CM-7, the serum

Table II. Serum TT_3, TT_4 levels in each experimental group (nmol/mL).

Group	TT_3	TT_4
Control	2.55 ± 0.55	39.12 ± 5.69
Hyperthyroidism	18.21 ± 3.21*	222.23 ± 44.21*
β-CM-7	10.11 ± 1.99#	131.12 ± 39.21#

"*" $p<0.05$ *vs*. control, "#" $p<0.05$ *vs*. Hyperthyroidism.

Table III. Left and right ventricular myocardial hypertrophy index.

Group	LVW/BW (mg/g)	RVW/BW (mg/g)
Control	0.78 ± 0.15	0.12 ± 0.04
Hyperthyroidism	1.41 ± 0.21**	0.43 ± 0.07**
β-CM-7	1.11 ± 0.09##	0.29 ± 0.04##

"*" $p<0.01$ *vs*. control, "#" $p<0.01$ *vs*. Hyperthyroidism.

TT_3 and TT_4 levels in mice were remarkably reduced, and the left and right ventricular MH indexes were significantly lower than those in the hyperthyroidism group. At the same time, Masson and HE staining revealed that the hyperthyroidism group had obvious fibrosis, disordered cell arrangement, and increased gaps, while the β-CM-7 group had markedly reduced myocardial fibrosis area and relatively aligned cells (Figure 1A and 1B).

β-CM-7 Relieved L-Thy-Induced Cardiac OS

MH will increase the OS response, leading to weakened anti-oxidative capacity of myocardial cells and accelerated cell damage. In this study, qRT-PCR (Figure 2A and 2B) and Western blot (Figure 2C) were carried out to detect the expressions of SOD1 and SOD2, which were related to anti-OS, in myocardial tissues. The results showed that compared with those in the control group, the mRNA and protein expressions of SOD1 and SOD2 in the myocardial tissues in the hyperthyroidism group were decreased dramatically, and they were significantly higher in the β-CM-7 group than those in the hyperthyroidism group. Besides, the total SOD, GSH-Px and MDA content was measured in the serum of the mice in each group (Figure 2D-2F). The results showed that compared with those in the control group, the levels of SOD and GSH-Px in the hyperthyroidism group were decreased dramatically, while the level of MDA was remarkably increased. Compared with those in the hyperthyroidism group, the levels of SOD and GSH-Px were increased, while the level of MDA was decreased dramatically in the β-CM-7 group. Furthermore, the ROS level in each group was tested, and it was found that the ROS level in the hyperthyroidism group was significantly increased, and β-CM-7 treatment dramatically inhibited the increase in ROS (Figure 2G).

β-CM-7 Relieved L-Thy-Induced Cardiac Inflammatory Response

IL-1β and TNF-α are the key indicators for inflammatory response. In this study, qRT-PCR (Figure 3A and 3B) and Western blot (Figure 3C) was performed to detect the expressions of IL-1β and TNF-α in myocardial tissues. The results showed that compared with those in the control group, the mRNA and protein expressions of IL-1β and TNF-α in the hyperthyroidism group were significantly increased, while they were dramatically lower in the β-CM-7 group than those in the hyperthyroidism group. Additionally, similar results were obtained from the detection of the expressions of IL-1β and TNF-α using

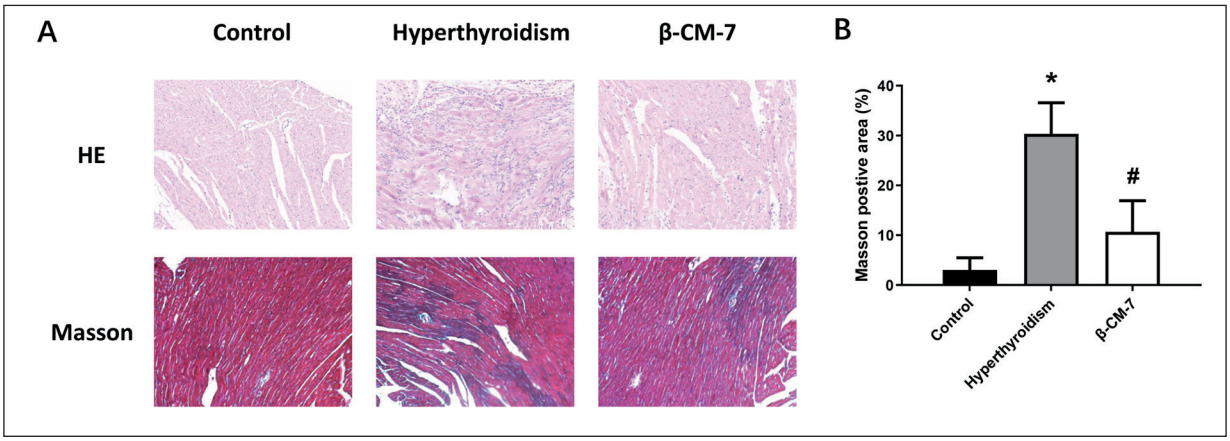

Figure 1. β-CM-7 relieved L-Thy-induced cardiac structural changes. **A,** HE staining and Masson staining (magnification: 200×). **B,** Masson staining analysis. ("*" indicates statistical difference from the Control group, $p<0.05$, and "#" indicated statistical difference from the hyperthyroidism group, $p<0.05$).

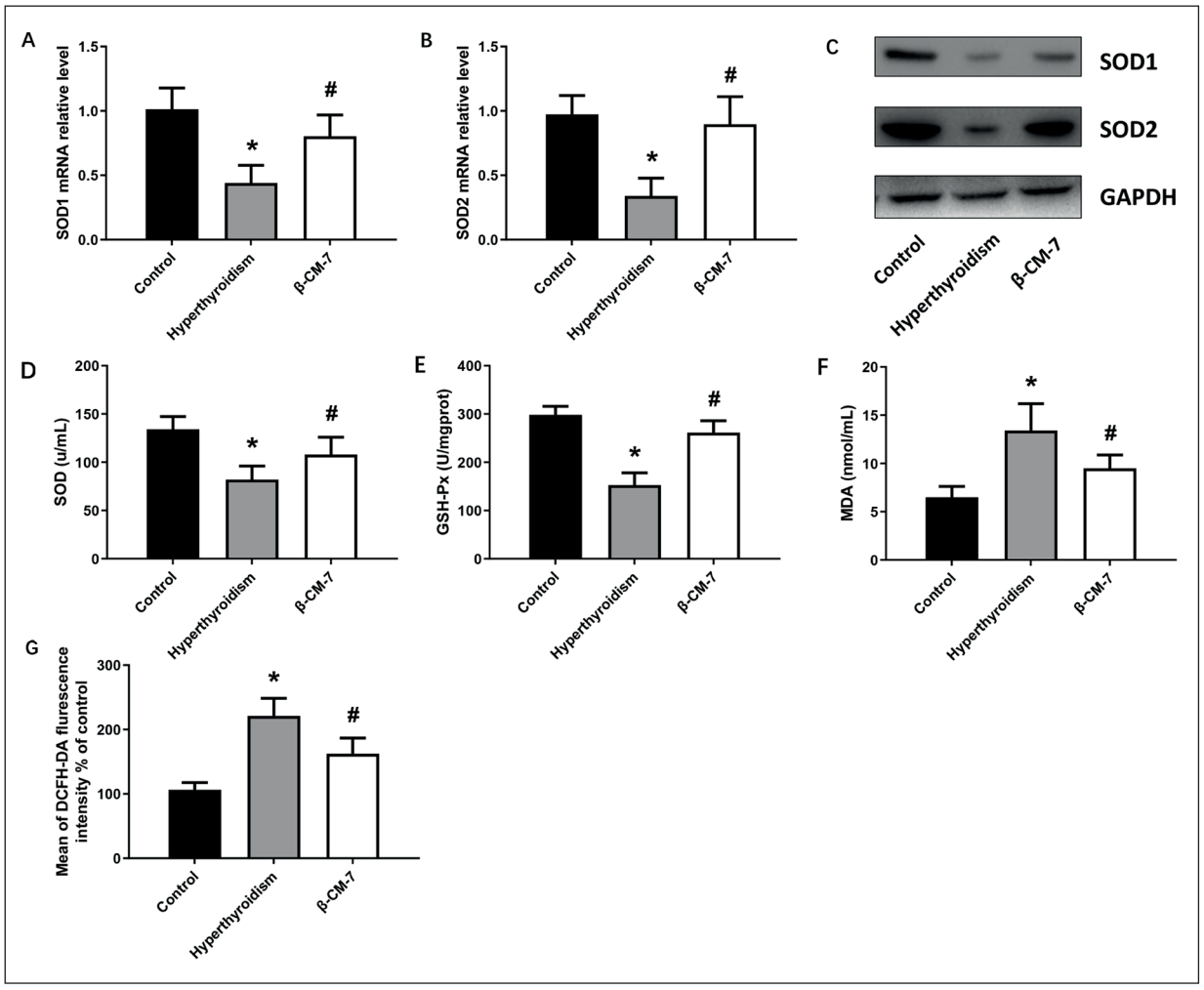

Figure 2. β-CM-7 relieved L-Thy-induced cardiac OS. **A,** qRT-PCR was used to detect SOD1 mRNA level. **B,** qRT-PCR detection of SOD2 mRNA level. **C,** SOD1 and SOD2 protein expressions. **D,** Detection of SOD activity. **E,** Detection of GSH-Px activity. **F,** Detection of MDA content. **G,** Flow detection of ROS levels. ("*" indicated statistical difference from the Control group, $p<0.05$, and "#" indicated statistical difference from the hyperthyroidism group, $p<0.05$).

ELISA (Figure 3D and 3E). Therefore, it was speculated that L-Thy-induced MH can induce inflammation, and β-CM-7 can inhibit inflammatory responses.

β-CM-7 Inhibited L-Thy-Induced NF-κB Signaling Pathway Activation

Studies have indicated that the NF-κB pathway has a certain regulatory effect on the occurrence and development of heart disease. Therefore, this conjecture was verified by treating H9c2 cells with L-Thy and β-CM-7. First, CCK-8 assay was conducted to find that 10^{-8} mol/L-10^{-6} mol/L β-CM-7 cultured for 6 h markedly increased cell viability (Figure 4A). Secondly, the expressions of p65, P-p65, and IkBα were tested. The Western blotting results showed that compared with those in the control group, the expressions of p65 and P-p65 in the model group were increased dramatically, but the expression of IkBα was decreased. The expressions of p65 and P-p65 in the β-CM-7 group were significantly lower than those in the model group, and the expression of IkBα was dramatically increased (Figure 4B), and qRT-PCR also obtained similar results (Figure 4C and 4D). At the same time, to clarify the change of p65 phosphorylation expression, immunofluorescence we applied to detect the change of P-p65 expression (Figure 4E). The results showed that p65 phosphorylation was increased remarkably

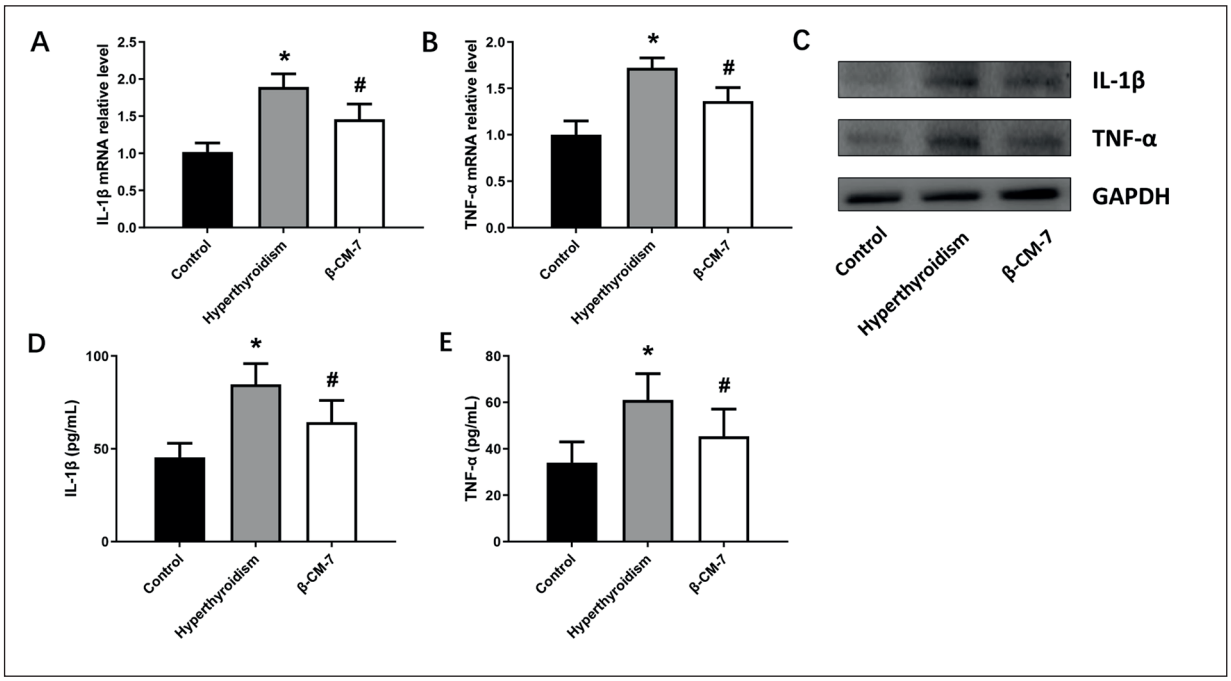

Figure 3. β-CM-7 relieved L-Thy-induced cardiac inflammatory response. **A,** qRT-PCR detected the level of IL-1β mRNA. **B,** qRT-PCR detection of TNF-α mRNA levels. **C,** IL-1β and TNF-α protein expressions. **D,** ELISA detection of IL-1β. **E,** ELISA detection of TNF-α content. ("*" indicated statistical difference from the Control group, $p<0.05$, and "#" indicated statistical difference from the hyperthyroidism group, $p<0.05$).

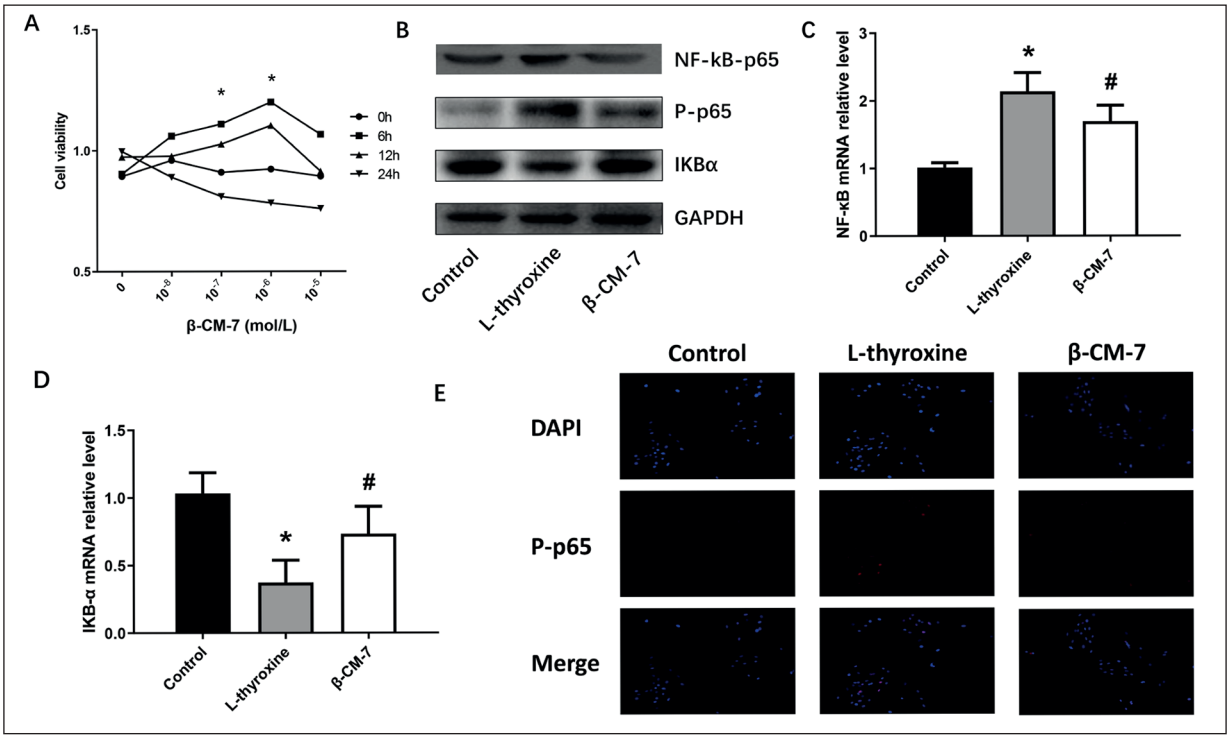

Figure 4. β-CM-7 inhibited L-Thy-induced NF-**κB** signaling pathway activation. **A,** CCK8 assay was used to detect the activity of H9c2 cells. **B,** NF-κB-p65, P-p65, IkBα protein expressions. **C,** qRT-PCR detection of NF-κB-p65 mRNA levels. **D,** qRT-PCR detection of IkBα mRNA levels. **E,** P-p65 expression was detected by immunofluorescence (magnification: 200×) ("*" indicated statistical difference from the Control group, $p<0.05$, and "#" indicated statistical difference from the L-thyroxine group, $p<0.05$).

in the model group, while P-p65 expression in the β-CM-7 group was decreased. Therefore, it was hypothesized that β-CM-7 could inhibit the over-activation of the NF-κB pathway, thereby protecting myocardial cells.

Discussion

Hyperthyroidism is a common clinical endocrine disease, which can affect multiple systems of the body, with corresponding clinical manifestations, which are manifested as increased food intake, weight loss, palpitations, sweating, irritability[11]. HHD is one of the special clinical manifestations of hyperthyroidism, which can not only aggravate the original cardiovascular disease, but also lead to arrhythmia alone, even heart failure or sudden death[12]. HHD can occur in any patient with hyperthyroidism, and its incidence accounts for about 10-20% of patients with hyperthyroidism, with an upward trend with age. In addition, MH is an independent risk factor for cardiovascular accidents. Patients with MH have a dramatically increased risk of arrhythmia and sudden death[4]. Therefore, preventing MH will effectively reduce the patient's condition.

This research revealed that the myocardial fibers of hyperthyroid mice were disordered, cardiomyocytes became hypertrophic and swollen, the horizontal stripes disappeared, and the nucleus was significantly enlarged. In addition, evident fibrosis and connective tissue hyperplasia in the interstitial tissue and thickened blood vessel walls were observed. The levels of serum TT_3 and TT_4 in the β-CM-7 group were dramatically lower than those in the model group, suggesting that the prevention and treatment effects of β-CM-7 on hypertrophic myocardium in hyperthyroid cardiomyopathy mice may be related to the reduction of TT_3 and TT_4 levels. Recent studies have shown that OS is also one of the important causes of structural and functional abnormalities of the cardiovascular system. A large amount of data has confirmed that OS is closely related to the formation of cardiac hypertrophy[13]. OS refers to a pathological state which results in the excessive accumulation of oxygen free radicals (OFR) and their related metabolites due to excessive generation of OFR and/or damage to the antioxidant defense system in the cell, resulting in a variety of toxic effects on cells[14]. The amount of OFR generated under physiological conditions is very few, which can regulate cell growth, gene expression, sterilization, and vasomotor contraction in the body, thereby playing an important physiological function. At the same time, the free radical removal system in the body can remove OFR in time. There are many types of anti-free radical substances in the body, mainly including all kinds of enzymatic and non-enzymatic antioxidants, such as superoxide dismutase (SOD)[15], glutathione peroxidase (GSH-Px), and multivitamins Vitamins[16]. These substances have specialized subcellular localization in the body, which can act on different links of OFR metabolism, reduce the production of OFR, or protect the cell membrane from attack, so as to prevent the damage of reactive oxygen species (ROS). In pathological conditions, when certain factors act on cells, the body's OFR is generated excessively or the antioxidant defense system is damaged, the dynamic balance of OFR production and removal is disrupted, and the rate of active oxygen production is greater than the rate of elimination. Then, the OFR will cause the accumulation of ROS, leading to the emergence of OS and further causing oxidative damage to macromolecules (mainly lipids, proteins and DNA). These injuries are the basis for aging, degenerative diseases, cardiovascular disease and tumor formation[17,18]. The results of this study also demonstrated that ROS and MDA were significantly increased in the model group, while SOD and GSH-Px expressions were dramatically reduced, but in the β-CM-7 group, SOD and GSH-Px expressions were increased, thereby eliminating excessive ROS and MDA. In recent years, some research shows that a small amount of ROS can regulate the normal physiological function of cardiomyocytes, but under the influence of hormones such as angiotensin, norepinephrine and TNF-α, NADPH oxidase is abnormally activated, generating excessive ROS, which leads to MH[19].

NF-κB is the most important transcriptional regulator that regulates OS and inflammatory pathways. The activated TLR4 activates the upstream inhibitory proteins IKKα and IkBα through a series of cascade reactions, causing the latter to phosphorylate and degrade, then activates NF-κB-p65 that is transferred into the nucleus and combines with the promoter of the pro-inflammatory gene, so as to increase gene expression and aggravate the inflammatory response, leading to body damage[20]. This study also

confirmed that the expressions of inflammatory factors IL-1β, TNF-α (*in vivo*), p65 and P-p65 (*in vitro*) were increased in the model group, while the expression of p65 (*in vitro*) upstream inhibitor IkBα was markedly reduced, which indicates that L-Thy-induced cardiac hypertrophy activated the NF-κB pathway, thereby promoting the expansion of the inflammatory response. In contrast, β-CM-7 treatment can effectively inhibit the excessive activation of the NF-κB pathway and inhibit the expression of downstream inflammatory factors, thereby alleviating the inflammatory response.

Previous studies have confirmed that oxygen free radicals participate in the development of myocardial hypertrophy, and this research indeed confirmed that β-CM-7 could inhibit inflammation and relieve redox imbalance in L-thyroxine-induced myocardial hypertrophy, thus providing experimental evidence for the treatment of hyperthyroid heart disease.

Conclusions

In this study it has been demonstrated that NF-κB pathway is involved in OS and inflammatory responses induced by HHD, while β-CM-7 may decrease cell damage by blocking the over-activation of NF-κB pathway, which may be an important target for HHD.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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