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Effects of carvedilol on expression of TLR4 and its downstream signaling pathway in liver tissue of rats with cholestatic liver fibrosis
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ABSTRACT
Objectives: This study was designed to investigate the effects of carvedilol on the expression of TLR4 and its downstream signaling pathway in liver tissue of rats with cholestatic liver fibrosis, and provided experimental evidence for clinical treatment of liver fibrosis with carvedilol.

Methods: A total of fifty male Sprague Dawley rats were randomly divided into five groups (10 rats per group): sham surgery control group, bile duct ligation (BDL) model group, low-dose carvedilol treatment group (0.1mg·kg⁻¹·d⁻¹), medium-dose carvedilol treatment group (1mg·kg⁻¹·d⁻¹), high-dose carvedilol treatment group (10mg·kg⁻¹·d⁻¹). Rat hepatic fibrosis model was established by applying BDL. Forty-eight hours after the operation, carvedilol was administered twice a day. The blood and liver were simultaneously collected under the aseptic condition for further detection in two weeks after operation.

Results: Compared with the sham group, the BDL group showed obvious liver injury,
increased levels of inflammatory factors, and continued progression of liver fibrosis. Carvedilol could alleviate the above changes. The improvement effects were augmenting as dosages increasing. In addition, compared with the BDL group, carvedilol can reduce the expressions of TLR4, MyD88 and NF-κB p65 in liver tissue and increase the expression of β-arrestin2, and the effect in the high dose group was more obvious.

**Conclusions:** Carvedilol can reduce the release of inflammatory mediators by down-regulating TLR4 expression and inhibiting its downstream signaling pathway, thus playing a therapeutic role in cholestatic liver fibrosis.

**Keywords:** Carvedilol; Liver fibrosis; TLR4; NF-κB; β-arrestin2

**INTRODUCTION**

Liver fibrosis refers to the abnormal proliferation of fibrous connective tissue in the liver caused by chronic inflammation or injury of the liver, which is a common pathological change of all chronic liver diseases. The common feature is increased synthesis of extracellular matrix (ECM) dominated by collagen, relatively insufficient degradation, and excessive deposition in the liver. If further development leads to the reconstruction of hepatic lobules, the formation of false lobules and nodules, it will enter the stage of cirrhosis and even liver cancer [1]. Among them, cholestasis is an important factor causing liver fibrosis. Long-term chronic cholestasis, due to the action of bile acid and bilirubin, causes hepatocyte degeneration, necrosis and liver fibrosis, and eventually leads to cirrhosis, which is pathologically known as biliary cirrhosis [2]. Whether it is primary biliary cirrhosis (PBC) or secondary biliary cirrhosis (SBC), there are currently no effective preventive and therapeutic drugs. Therefore, active development of new drugs for the prevention and treatment of cholestatic liver fibrosis is a hot spot in current medical research.

The liver is the largest and most important immune organ in the human body, and almost all factors leading to liver cell damage are ultimately related to immune
factors, while Toll-like receptors (TLRs) are bridges connecting the innate and adaptive immunity of human body and play an important role in the non-specific immunity and specific immunity of the body. TLR4 is the most widely studied member of the TLRs family in liver diseases. It can specifically recognize pathogen-associated molecular patterns (PAMPs), recruit myeloid differentiation factor (MyD88), activate TLR4/NF-κB signaling pathway, promote the release of downstream inflammatory factors, and eventually cause inflammatory response. Carvedilol is a new generation of non-selective β-blocker used for vasodilating and antagonism at α1. Its beneficial role in myocardial infarction and chronic heart failure has been confirmed by large-scale clinical trials. It has been found that carvedilol can inhibit the synthesis of ECM in order to alleviate cardiac interstitial remodeling and prevent the occurrence and development of heart failure. However, there are few reports discussing whether Carvedilol can effectively inhibit hepatic fibrosis as well as its underlying pharmacological mechanism. In this experiment, the effects of carvedilol on the expression of TLR4 and its downstream signaling pathway in liver tissues during cholestatic liver fibrosis were studied, and the possible mechanism of carvedilol inhibiting liver fibrosis was discussed. The research results will be of great significance in preventing and treating liver fibrosis, and also have a strong clinical application value.

MATERIALS AND METHODS

Animal models

Fifty adult male Sprague Dawley rats weighing 250-300g were obtained from the Experimental Animal Center of Hebei Medical University (Permission No.: 705188). The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication #85-23, revised in 1985). The experiment was performed in compliance with the national ethical guidelines for the care and use of laboratory animals (certificate no. 911102). They were randomly divided into five groups (10 rats per group): sham surgery control group, bile duct ligation (BDL) model group, low-dose carvedilol treatment
group (0.1mg·kg⁻¹·d⁻¹, CAR-L), medium-dose carvedilol treatment group (1mg·kg⁻¹·d⁻¹, CAR-M), high-dose carvedilol treatment group (10mg·kg⁻¹·d⁻¹, CAR-H). Rat hepatic fibrosis model was established by applying BDL. Forty-eight hours after the operation, carvedilol was administered twice a day. The blood and liver were simultaneously collected under the aseptic condition for further detection in two weeks after operation.

Liver function detection and histological examination
Blood was obtained from the left ventricular apex of rats and centrifuged at 3000 g at 4°C for 10 min to collect the serum. The level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBil) and albumin (Alb) were detected using a fully automatic biochemical analyzer. Liver specimens were fixed 12-24 h in 4% phosphate-buffered paraformaldehyde (Huarui Scientific & Technological Co.) and then embedded in paraffin for light microscopy examination. Tissue sections (4-μm thick) were stained with hematoxylin and eosin (H&E) for morphological evaluation and Masson trichrome (MT) for assessing the degree of fibrosis.

Hydroxyproline determination
Collagen was detected by estimating the hydroxyproline content, an amino acid characteristic of collagen. Hepatic hydroxyproline was measured using a hydroxyproline detection kit (Jiancheng Institute of Biotechnology, Nanjing, China) according to the manufacturer’s instructions.

Western blot analysis
Liver samples were homogenized with lysis buffer (Cell Signal Technology Inc., Danvers, MA) and centrifuged at 20,000*g for 60 minutes at 4°C. The resultant supernatants were used as the total liver protein and subjected to Western blotting. The protein concentration in the supernatant was determined according to Bradford’s method. Proteins were resolved by sodium dodecyl sulphate polyacrylamide gel electrophoresis and transferred to a polyvinyl difluoride
membrane. The blots were probed with primary antibodies against TLR4 (Abcam), NF-κB p65 (Abcam), β-arrestin2 (Abcam), and GAPDH (Cell Signaling Technology), followed by a secondary antibody, and their reaction products were quantified by Quantity One software (Bio-Rad Laboratories, Inc., Berkeley, CA). The relative protein expression was identified as the ratio of gray value of TLR4, NF-κB p65 and cleaved caspase-3 bands to that of GAPDH band.

**Realtime-PCR analysis**

Quantitative analysis of TLR4, MyD88, TNF-α and IL-6 mRNA was performed by Realtime-PCR. Total RNA was extracted by TRIzol reagent from Sigma. Purity and concentration of RNAs were determined by a NaNO2000 UV spectrophotometer and OD260/OD280 was between 1.8~2.2, which indicated the high purity of RNAs. According to the manufacturer’s instructions, 1μg RNA was primed with oligo (dT) using a reverse transcriptase kit from Promega. The cDNA production was amplified in an qPCR instrument from Roche. The conditions for amplification were as follows: pre-denaturation for 30s at 95°C for 1 cycle; denaturation for 10s at 95°C, annealing for 30s at 58°C and extension for 30s at 72°C for a total of 40 cycles of PCR. Three replicates were prepared for each sample in PCR detection and data were analyzed by the ²⁻¹⁰⁰⁰ method, where ΔCt= Ct target gene-Ct reference gene, and ΔΔCt=ΔCt test group -ΔCt control group.

**Statistical analysis**

The data was expressed as means ± standard deviation (SD). Quantitative variables among groups were compared with one-way ANOVA, post-hoc test. A p value < 0.05 was considered significant. All statistical analyses of the experimental data were performed with SPSS 19.0 software (Chicago, IL, United States).

**RESULTS**

**Assessment of general health**

After 1 h to 2 h of BDL, the rats resumed their activities, and their urine turned yellow around 48 h. After 3 to 4 days, their skin and hair turned yellow, their spirit became worse, their food intake was less than that of the sham group, and their
weight gain was not obvious or slightly decreased. After 8 to 9 days, the rats gradually developed lethargy, obvious jaundice, and off-white feces, as well as had its reaction retarded and activity decreased. After 20 days, the intake of food was significantly reduced, and the body weight was significantly reduced compared with the sham group, and some rats gradually developed abdominal bulge.

**Carvedilol reduced liver injury**

We found that the liver functions in hepatic fibrosis model groups were obviously damaged according to the expression of ALT, AST, TBIL and Alb. And the liver function in carvedilol-treated groups were significantly better than that in BDL model group. (Table 1)

**Carvedilol attenuated liver fibrosis**

HE and MT staining were used to evaluate the therapeutic effects of different dosages of carvedilol to hepatic fibrosis. After 2 weeks of the operation, the liver tissues in the BDL group exhibited distortion architecture, showing that hepatocellular degeneration with formation of fibrous tissue infiltrated with inflammatory cells. Liver specimens obtained from carvedilol-treated group displayed a significant decrease in fibrous tissue and the hepatocytes nearly remained their normal appearance, especially in the CAR-H group. (Figure 1) As determined by Masson's trichrome staining, BDL markedly induced the liver fibrosis. These alterations were significantly attenuated by carvedilol administered, and efficacy was obvious as dosage increasing. (Figure 2)

**Carvedilol prevented the abnormal collagen deposition**

Hydroxyproline is unique to collagen and thus serves as a specific biochemical marker of collagen production. 2 weeks after BDL, hydroxyproline content in the BDL group was markedly higher than that in the sham group (0.362±0.039μg·mg⁻¹ vs 0.186±0.021μg·mg⁻¹; P<0.01). However, it was significantly decreased in CAR-M and CAR-H groups (0.323±0.042μg·mg⁻¹, 0.289±0.038μg·mg⁻¹ vs 0.362±0.039μg·mg⁻¹; P
Carvedilol reduced TLR4 expression

Compared with the sham group, the expressions of TLR4 protein (0.47±0.14 vs 0.15±0.06; P<0.01) and mRNA (1.62±0.44 vs 0.48±0.20; P<0.01) increased significantly in the BDL group. Medium and high doses of carvedilol significantly reduced the expressions of TLR4 protein (0.34±0.11, 0.29±0.11 vs 0.47±0.14; P<0.05, P<0.01, respectively) and mRNA (0.96±0.29, 0.71±0.26 vs 1.62±0.44; P<0.01, P<0.01, respectively). No significant difference were also found between CAR-L group and BDL group (P>0.05). (Figure 4 A, B) (Figure 5)

Carvedilol reduced the expressions of MyD88 and NF-κB p65

MyD88 is an adaptor protein of TLR4 and a link protein of NF-κB. Some extracellular inflammation signals can be transmitted to MyD88 via TLR4 and then initiate downstream signaling pathways. 2 weeks after BDL, the expression of MyD88 mRNA in the BDL group was markedly higher than that in the sham group (1.57±0.41 vs 0.55±0.23; P<0.01). However, it was significantly decreased in CAR-M and CAR-H groups (1.19±0.31, 0.78±0.29 vs 1.57±0.41; P<0.05, P<0.01, respectively). (Figure 5)

Western blot analysis showed that the expression of NF-κB p65 in the BDL group was markedly higher than that in the sham group (0.54±0.16 vs 0.19±0.07; P<0.01). However, it was significantly decreased in CAR-L, CAR-M and CAR-H groups (0.43±0.12, 0.35±0.13, 0.30±0.11 vs 0.54±0.16; P<0.05, P<0.01, P<0.01, respectively). (Figure 4 A, C)

Carvedilol reduced inflammatory factors levels

We measured the levels of inflammatory factors in liver tissue, and the results showed that the expression of TNF-α mRNA in the BDL group was markedly higher than that in the sham group (1.47±0.38 vs 0.31±0.17; P<0.01). However, it was significantly decreased in CAR-L, CAR-M and CAR-H groups (1.18±0.34, 0.91±0.28, 0.68±0.25 vs 1.47±0.38; P<0.05, P<0.01, P<0.01, respectively). In addition, the
expression of IL-6 mRNA in BDL group was markedly higher than that in sham group (1.32±0.41 vs 0.27±0.16; \( P<0.01 \)). However, it was significantly decreased in CAR-M and CAR-H groups (0.98±0.31, 0.75±0.25 vs 1.32±0.41; \( P<0.05, P<0.01 \), respectively). (Figure 6)

**Carvedilol increased the expression of β-arrestin2**

β-arrestin2 is widely expressed in mammalian tissues. It is a multifunctional framework protein and plays an important role in signal transduction pathway of G protein-coupled receptor. Western blot analysis showed that the expression of β-arrestin2 in the BDL group was markedly lower than that in the sham group (0.18±0.10 vs 0.46±0.11; \( P<0.01 \)). However, it was significantly increased in CAR-L, CAR-M and CAR-H groups (0.29±0.11, 0.35±0.14, 0.41±0.13 vs 0.18±0.10; \( P<0.05, P<0.01, P<0.01 \), respectively). (Figure 4 A, D)

**DISCUSSION**

Liver fibrosis is a common pathological process of various chronic liver diseases, and it is a necessary stage to develop liver cirrhosis and primary liver cancer. Many years of medical research has found that liver fibrosis can be reversed \(^7\). Therefore, prevention and reversal of liver fibrosis has become an important link in the prevention and treatment of liver cirrhosis and liver cancer. BDL method is commonly used to induce liver fibrosis model. It has a short molding time, good repeatability, low requirements on animals, and its pathogenic factors are very similar to clinical cholestatic liver fibrosis. Therefore, this model is of great value in the evaluation of efficacy in the prevention and treatment of liver fibrosis, especially cholestatic liver fibrosis. In addition, it is also a powerful tool for studying hepatic encephalopathy and portal hypertension\(^8,9\). In this study, BDL method was used to establish the model of cholestatic liver fibrosis. Two weeks after operation, the liver function showed that ALT, AST and TBIL increased significantly, suggesting that liver function was damaged significantly. HE and MT staining and determination of hydroxyproline content showed collagen deposition in liver tissue and progress of liver fibrosis, suggesting that the model was successfully established.
TLRs are one of the cellular transmembrane receptors and pathogen pattern recognition receptors in the innate immune system. They can recognize the PAMPs of many pathogenic microorganisms and are widely distributed in liver, spleen, lung, heart, kidney, brain, thymus and other tissues of animals. TLRs are mainly involved in the identification of pathogenic microbial products and inflammatory signal transduction, and are bridges connecting innate immunity and acquired immunity [10]. TLRs in the liver can be exposed to a large number of pathogen components through the portal vein, and are widely involved in the physiological and pathological processes of the liver through signal transduction [11]. In recent years, the role of TLRs in some liver diseases such as viral hepatitis, cirrhosis, liver transplantation and autoimmune hepatitis has been preliminarily recognized [12]. With the further study of TLRs, some scholars have found that hepatic stellate cell (HSC) membrane also expresses TLRs, which means that TLRs may be involved in the occurrence and development of hepatocyte injury and liver fibrosis [13]. At present, TLR4 is widely studied in the field of liver fibrosis. TLR4 is the earliest found in mammalian TLRs. It has been confirmed that lipopolysaccharide, the main component of endotoxin, is the main ligand of TLR4 [14]. After binding to endotoxin, TLR4 promotes the release of various inflammatory factors such as tumor necrosis factor and oxygen free radicals, and induces the generation and activation of HSC. Activated HSC utilize components of TLR4 signal transduction cascade to stimulate NF-κB and up-regulate chemokines and adhesion molecules, thus triggering the development of liver fibrosis [15,16]. Therefore, it is of great theoretical and clinical significance to investigate the changes of TLR4 expression in liver during the course of liver fibrosis. This study confirmed that the expression of TLR4 was low in the sham group, but the levels of TLR4 mRNA and protein in the BDL group were significantly up-regulated with the development of liver fibrosis, suggesting that TLR4 is involved in the process of liver fibrosis.

TLRs recognize the corresponding PAMPs and initiate downstream signal transduction pathways. The most important adaptor protein is MyD88. Through TIR and linkage between TLRs/IL-1R, MyD88 transmits signals into the cells, which
activates the transcription factors such as NF-κB, causing it to release a variety of inflammatory cytokines as well as anti-apoptotic molecules, ultimately contributing to the inherent immunity of the human body \[17\]. NF-κB is an important transcription factor that widely exists in the body to regulate the expression of various inflammation- and immune-related genes. The main form of NF-κB is dimer P65/P50 with a transcription activity binding site only in the C-terminal of p65 \[18\]. So the amount of activated NF-κB can be determined by directly measuring NF-κB p65. NF-κB p65 is activated and translocated into the nucleus, where it binds to the κB motif in the promoter or enhancer of the target genes, to induce the transcription of many factors \[19\]. The expression of most inflammatory mediators, such as TNF-α, IL-6 is regulated by NF-κB during the inflammatory response. TNF-α is an inflammatory mediator with a variety of biological activities, which can play a role as a key cytokine in the processes of inflammation and immunity in various parts of the body. Several studies have shown that TNF-α can promote the activation and proliferation of HSC and the deposition of ECM, ultimately leading to liver fibrosis \[20-23\]. IL-6 also has a variety of biological activities, which can regulate the proliferation and differentiation of cells and the expression of specific genes, and also plays an important role in the process of liver fibrosis \[24,25\]. These findings were also confirmed in this study. In the BDL group, the expression of MyD88 in the liver was significantly increased, NF-κB was overactivated, and a large number of inflammatory mediators such as TNF-α and IL-6 were released, which were linearly correlated with the expression of TLR4, suggesting that the excessive activation of TLR4/MyD88/NF-κB pathway in cholestatic rats was involved in the occurrence and development of liver fibrosis. Therefore, effectively inhibiting the activity of TLR4 and its downstream signaling pathway may be an effective means to inhibit and reverse liver fibrosis and achieve the purpose of liver protection.

Carvedilol is currently used in gastroenterology department to reduce portal hypertension in patients with cirrhosis. Its efficacy and drug tolerance are superior to that of propranolol, a traditional antihypertensive agent that lowers portal vein pressure. It has a good prospect in the treatment of patients with liver cirrhosis. In
In this study, it was confirmed that carvedilol can improve the liver function of cholestatic rats, reduce collagen deposition and inhibit the occurrence and development of liver fibrosis, and the effect was more obvious in the high-dose group. In addition, carvedilol can decrease the expressions of TLR4 and MyD88, reduce the activation of NF-κB, and inhibit the release of TNF-α and IL-6. Therefore, we speculated that the inhibition of liver fibrosis by carvedilol is related to the inhibition of the TLR4/MyD88/ NF-κB signaling pathway. In previous studies of the heart, it was shown that carvedilol can inhibit NF-κB activity by activating the β-arrestin2 signaling pathway [24]. There are two signaling pathways on the G-protein coupled receptor, the traditional G protein signaling pathway and the β-arrestin2 signaling pathway [27]. When carvedilol inhibits the β2 adrenergic receptor (β2AR), it only inhibits the traditional signaling pathway, and has a promoting effect on the β-arrestin2 signaling pathway instead of inhibiting it. James et al. [28] demonstrated that carvedilol could promote β2AR phosphorylation and recruited β-arrestin2 to phosphorylated β2AR to activate the β-arrestin2 signaling pathway. β-arrestin2 can also inhibit the activity of NF-κB by preventing the degradation of IκBα, a blocker of NF-κB [29]. In the experiment of β2AR agonist stimulating monocytes, Wang et al. [30] found that the expression of β-arrestin2 in the cell membrane increased under the action of agonists, and the TLR4 pathway was inhibited. When the β-arrestin2 gene was deleted by siRNA technology, the inhibitory effect of agonists on TLR4 pathway disappeared. In this study, it was found that the expression of β-arrestin2 decreased significantly and the expression of NF-κB p65 increased significantly in BDL rats. Carvedilol can significantly increase the expression of β-arrestin2 and decrease the expression of NF-κB p65, suggesting that carvedilol can affect the activity of NF-κB by regulating β-arrestin2. Although we found that carvedilol can inhibit the expression of TLR4, the mechanism of carvedilol inhibiting TLR4 expression is not clear. Whether carvedilol can directly inhibit the expression of TLR4 or inhibit the expression of TLR4 by blocking the expression of NF-κB can not be proved, which needs further study.

In conclusion, carvedilol can reduce the release of inflammatory mediators and inhibit the progression of liver fibrosis by down-regulating the expression of TLR4
and inhibiting the TLR4/MyD88/NF-κB signaling pathway, and exhibit dose-effect relationship. Although the specific molecular mechanisms involved in this process require further study, we believe that inhibiting TLR4 and its downstream signaling pathway will provide effective targets for the prevention and treatment of cholestatic liver fibrosis.

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CONFLICTS OF INTEREST
All authors have read the journal’s policy on the disclosure of potential conflicts of interest and have none to declare. All authors have read the journal’s authorship agreement. All authors have read the manuscript and approved of its submission for publication.

REFERENCES


### Table 1. Impact of carvedilol on ALT, AST, ALB and BIL in BDL rats (mean± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT(U/L)</th>
<th>AST(U/L)</th>
<th>ALB(g/L)</th>
<th>TBil(μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>46.9±4.37</td>
<td>131.1±12.23</td>
<td>35.2±2.57</td>
<td>1.7±0.41</td>
</tr>
<tr>
<td>BDL</td>
<td>93.1±11.35*</td>
<td>422.3±42.61*</td>
<td>28.5±4.02*</td>
<td>86.8±12.18*</td>
</tr>
<tr>
<td>CAR-L</td>
<td>90.5±9.15*</td>
<td>403.7±36.48*</td>
<td>27.8±2.45*</td>
<td>85.4±9.52*</td>
</tr>
<tr>
<td>CAR-M</td>
<td>84.8±14.23*</td>
<td>389.8±47.62*</td>
<td>27.9±3.34*</td>
<td>82.1±12.87*</td>
</tr>
<tr>
<td>CAR-H</td>
<td>80.6±11.23*#</td>
<td>368.8±40.52*#</td>
<td>28.3±3.18*</td>
<td>77.9±13.56*</td>
</tr>
</tbody>
</table>

BDL, bile duct ligation; SHAM, sham-operated; CAR, Carvedilol; ALT, alanine transaminase; AST, aspartate transaminase; ALB, albumin; TBil, total bilirubin. *P <0.05 vs SHAM; #P <0.05 vs BDL.
Figure 1. HE staining of histological section was performed to evaluate pathological changes (200×). A: SHAM group, hepatic cords were well arranged, the structure of hepatic lobule was intact; B: BDL group, disappeared normal arrangement of hepatic plates, disordered lobule structure; C: CAR-L group; D: CAR-M group; E: CAR-H group. The pathological change of liver in the carvedilol groups was rather lighter compared with the model after 2 wk of BDL, the degrees of the effects were carvedilol dose-dependent.

Figure 2. Masson staining of histological section of hepatic tissue (200×). A: SHAM group, Liver tissue showed normal lobular architecture and a normal distribution of collagen with a thin rim around central veins; B: BDL group, Liver
tissue showed fibrous tissues around portal areas, enlarging portal areas, fibrous tissues proliferation also in hepatic lobule; C: CAR-L group; D: CAR-M group; E: CAR-H group. Liver tissue from carvedilol group showed marked reduction in collagen deposition compared with the model after 2 wk of BDL, the degrees of the effects were carvedilol dose-dependent.

Figure 3. The content of hydroxyproline in different groups.
Liver tissue from carvedilol group showed marked reduction in the content of hydroxyproline compared with those of the BDL model group, the degrees of the effects were carvedilol dose-dependent. ▲ P<0.01, △ P<0.05 vs BDL model group

Figure 4A: Expressions of TLR4, NF-κB p65 and β-arrestin2 in the liver tissue in different groups (Western blot analysis).

Figure 4B: The expression of TLR4 increased significantly in the BDL group. Carvedilol can obviously reduce the expression of TLR4 compared with those of the BDL model group, the degrees of the effects were carvedilol dose-dependent.
**Figure 4C:** The expression of NF-κB p65 increased significantly in the BDL group. Carvedilol can obviously reduce the expression of NF-κB p65 compared with those of the BDL model group, the degrees of the effects were carvedilol dose-dependent.

**Figure 4D:** The expression of β-arrestin2 decreased significantly in the BDL group. Carvedilol can obviously increase the expression of β-arrestin2 compared with those of the BDL model group, the degrees of the effects were carvedilol dose-dependent. ▲ P<0.01, △ P<0.05 vs BDL model group.

**Figure 5.** Realtime-PCR analysis showed that the expressions of TLR4 and MyD88 in the BDL group were significantly higher than that in the sham group. The expressions of TLR4 and MyD88 were markedly decreased after carvedilol treatment, especially in the CAR-M and CAR-H groups. ▲ P<0.01, △ P<0.05 vs BDL model group.
Figure 6. Realtime-PCR analysis showed that the expressions of TNF-α and IL-6 in the BDL group were significantly higher than that in the sham group. The expressions of TNF-α and IL-6 were markedly decreased after carvedilol treatment, especially in the CAR-M and CAR-H groups. ▲P<0.01, △P<0.05 vs BDL model group.