Research Article

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Effects of the interaction of Notch and TLR4 pathways on inflammation and heart function in septic heart

Abstract: We investigated the role of the interaction between the Notch and Toll-like receptor 4 (TLR4) pathways in septic myocardial injury. The sepsis model was induced in rats with lipopolysaccharide (LPS). Rats were divided into control, LPS, LPS + TAK242 ((6R)-6-[N-(2-chloro-4-fluoro-phenyl)sulfamoyl]cyclohex-1-ene-1-carboxylate) and LPS + DAPT (N-[N-(3,5-difluorophenacetyl)-L-alanyl]-s-phenylgly-cinetbutylester) groups. Heart function was evaluated with a Cardiac Doppler ultrasound. Myocardial morphological changes were detected by hematoxylin-eosin staining (H&E). Apoptosis was assessed by a TUNEL assay. The mRNA and protein levels were detected with real-time PCR, Western blot, and immunohistochemistry analysis. We found that heart function in the LPS + TAK242 group was significantly improved, but not in the LPS + DAPT group. LPS + TAK242 had a lower level of degeneration and necrosis of cardiomyocytes and inflammatory cell infiltration, as well as lower apoptosis and caspase-3 expression than the LPS group. Compared with the LPS group, the inflammatory cell infiltration was reduced in the LPS + DAPT group, while the degeneration and necrosis of cardiomyocytes were not obviously improved.

Additionally, the expression levels of tumor necrosis factor-α and Interleukin-6, the protein contents of Notch intracellular domain and Hes1, and the P65 nuclear factor kappa-B (NF-κB) to P-P65 NF-κB ratio in LPS + TAK242 group and LPS + DAPT group were significantly lower than those in LPS group. Conclusively, the interaction between TLR4 and Notch signaling pathways enhances the inflammatory response in the septic heart by activating NF-κB. Blocking the TLR4 pathway with TAK242 can improve heart dysfunction and myocardial damage in sepsis, while blocking the Notch pathway with DAPT cannot effectively prevent heart dysfunction and myocardial damage in sepsis.

1 Introduction

Sepsis is defined as the life-threatening multiple organ dysfunction caused by the imbalance of the host response to infection [1], which has been recognized as the main cause of death in intensive care units [2]. Currently, the in-hospital sepsis and septic shock mortality remains as high as 20–30% [3]. Patients with sepsis may suffer from various degrees of heart dysfunction [4], and in severe cases, sepsis may lead to multiple organ failure [5]. Heart dysfunction in sepsis is associated with significantly increased mortality. When patients with sepsis have heart dysfunction, the mortality rate may be significantly increased by 20–50% [6]. In sepsis, the toll-like receptors (TLRs), inflammasomes, and other pattern recognition receptors would initiate the immune responses after recognizing the pathogen-associated molecular patterns derived from microorganisms [7]. Lipopolysaccharide (LPS), which is a pathogen-associated molecular pattern [8,9], is recognized by TLR4. It can trigger immune responses and act as an early signal of pathogenic microbial infection [10,11]. LPS is the main component of the cell wall of gram-negative bacteria [12] and has been widely

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used for inducing sepsis models [13,14]. However, there is no specific effective treatment for sepsis patients; therefore, sepsis has been mainly treated by controlling the infection with antibiotics and supporting the organ function [15,16]. In sepsis, how to reduce severe myocardial damage and the occurrence of heart dysfunction have attracted much attention.

After activation, TLR4 can induce inflammation [10,12,17–19] and the expression of nuclear factor kappa-B (NF-κB)-dependent pro-inflammatory cytokines, such as the tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) [20,21]. Many studies have shown that the inflammatory response induced by the TLR4 pathway plays an important role in myocardial injury caused by sepsis [22–25]. On the other hand, after the Notch receptor binds to its ligand, its transmembrane domain would be cleaved by the γ-endocrine enzyme complex to release the intracellular active form of Notch intracellular domain (NICD), which enters the nucleus to interact with target genes and induce the transcription of Notch target genes (such as the Hairy and Enhancer of Split 1 [Hes1] gene) [26–28]. There is crosstalk between the Notch and TLR4 signaling pathways. The activated TLR4 signal may directly regulate the Notch cascade through the histone modification at the target gene sites of Notch [29] or indirectly regulate the Notch cascade by inducing the Notch receptors and ligands [30,31]. Many studies have shown that the Notch signaling can enhance the TLR4-related inflammatory responses, both *in vitro* and *in vivo*, and the inflammatory response would be declined after inactivating the Notch signaling [29,31,32]. The NOTCH pathway can increase the expression of cytokines in the heart and strengthen the inflammatory responses in a mouse model of myocardial ischemia, but blocking the NOTCH pathway does not reduce the area of myocardial infarction [33]. However, the roles of the Notch pathways in septic myocardial injury need to be further studied.

This study investigated the roles of TLR4 and Notch signaling in septic myocardial injury and the effects of the interaction between TLR4 and Notch on septic heart tissue. The sepsis model was induced by LPS. The TLR4 and Notch signaling pathways were, respectively, blocked with (6R)-6-[N-(2-chloro-4-fluorophenyl)sulfamoyl]cyclohex-1-ene-1-carboxylate (TAK242) and N-[N-(3,5-difluorophenacetyl)-L-alanyl]-s-phenylglycinetbutylester (DAPT). The heart function, pathological damage, inflammation level in septic heart, and the key proteins of TLR4 and Notch signaling were evaluated. Our findings may help identify novel mechanisms for sepsis myocardial

injury and cardiac dysfunction and provide therapeutic targets for the disease treatment.

2 Methods

2.1 Animals

Totally, 24 male SD rats (7–8-weeks-old; weighing 180–200 g) were provided by the Animal Experiment Center of Xinjiang Medical University. All animals were individually housed at room temperature (22–24°C) under standard conditions, with a light/dark cycle of 12:12 h. Animal experiments started after 7 days of acclimatization to the environment. All animals were subjected to free access to food and water but fasted for 12 h before the experiments.

Ethical approval: The research related to animal use has been complied with all the relevant national regulations and institutional policies for the care and use of animals, and were approved by the Ethics Committee of the Animal Experiment Center of Xinjiang Medical University (Ethics approval No.: IACUC20200924-21).

2.2 Animal modeling

The SD rats were randomly divided into control, LPS, LPS + TAK242, and LPS + DAPT groups, with eight rats in each group. In the control group, rats received an intraperitoneal injection of 10% DMSO + 90% corn oil. In the LPS group, rats were injected intraperitoneally with 10% DMSO + 90% corn oil and LPS (15 mg/kg; derived from the *Escherichia coli* [serum type: Coli O55:B5]) to induce sepsis [34]. Rats in the LPS + TAK242 group received intraperitoneal injection of TAK242 (3 mg/kg; dissolved in 10% DMSO + 90% corn oil; MedChemExpress, USA) [35] and LPS (15 mg/kg). For rats in the LPS + DAPT group, they received intraperitoneal injection of DAPT (100 mg/kg; dissolved in 10% DMSO + 90% corn oil; MedChemExpress, USA) [36] and LPS (15 mg/kg). The TAK242 and DAPT injections were conducted 3 h before LPS stimulation. At 14 h after injection, the rats were subjected to anesthesia with an intraperitoneal injection of sodium pentobarbital (1%; 40 mg/kg body weight), followed by echocardiography. Tissue specimens were collected immediately after the echocardiography.

2.3 Cardiac Doppler ultrasound examination

A high-frequency RMV707B high-frequency ultrasound probe (Visual Sonics, Toronto, ON, Canada) was used for the cardiac function assessment. All the images were collected by an experienced operator who was blinded to the experimental design. M-mode echocardiograms were collected from the long and short axis papillary muscles of the parasternal left heart. The left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), ejection fraction (EF), and fractional shortening (FS) were calculated.

2.4 Western blot analysis

The heart apical tissue was lysed for protein extraction. Totally 100 µg of total protein was separated by SDS-PAGE and then electronically transferred onto the PVDF membrane. After blocking with 10% non-fat milk at room temperature for 2 h, the membrane was treated with the primary antibodies against GAPDH (1:10,000 dilution; Abcam, Cambridge, UK), NICD (1:1,000 dilution; Abcam), NF-κB P65 (1:1,000 dilution; Abcam), P-NF-κB P65 (1:1,000 dilution; Abcam), Hes1 (1:1,000 dilution; Abcam), TNF-α (1:1,000 dilution; Abcam), IL-6 (1:1,000 dilution; Affinity Biosciences, China), and caspase-3 (1:2,000 dilution; Abcam), respectively, at 4°C overnight. Then, the membrane was treated with goat anti-rabbit IgG H&L (HRP) (1:20,000, Abcam) at 37°C for 2 h. Protein bands were developed with the ECL kit (Boster, Wuhan, Hubei, China) and analyzed with the Image Lab system. GAPDH was used as an internal reference.

2.5 Quantitative real-time PCR

Total RNA was obtained with the RNA isolation kit (Tiangen, Beijing, China) from the apex of the heart. Then, the total RNA was subjected to reverse transcription using the FastKing RT kit (Tiangen). The quantitative real-time PCR was performed with the SuperReal PreMix Plus (SYBR Green) (Tiangen) on the Quantstudio™ 6 Flex real-time PCR system (Thermo-Fisher Scientific, Waltham, MA, USA). The primers were all purchased from Bomed (Beijing, China), and the sequences were as follows: *GAPDH*, forward 5′-TTGTGCAGTGCCAGCCTC-3′ and reverse 5′-GAAGGGGTCGTTGATGGCAA-3′; *TNF-α*, forward 5′-ATGG GCTCCCTCTCATCAGT-3′ and reverse 5′-GCTTGGTGGTTTGC

TACGAC-3′; and *IL-6*, forward 5′-CTTGGAAATGAGAAAA GAGTTGTGC-3′ and reverse 5′-ACGGAACTCCAGAAGAC CAG-3′. PCR conditions were set as follows: 95°C for 15 min; 95°C for 10 s, and 55°C for 30 s, for 40 cycles. The 2^{-DDCT} method was used to analyze the relevant expression levels of target genes. GAPDH was used as an internal reference.

2.6 H&E staining

The heart tissues were fixed with 4% paraformaldehyde for 48 h and then embedded in paraffin. After deparaffinization, the tissues were made into sections and stained with hematoxylin and eosin (H&E) according to the routine procedure. The degree of inflammatory cell infiltration and cardiomyocyte damage was assessed.

2.7 Immunohistochemistry

The deparaffinized tissue sections were incubated with 3% H_2O_2 at room temperature for the immunohistochemical staining. After blocking with goat serum, the sections were treated with the primary antibodies against NICD (1:400 dilution; Abcam), NF-κB P65 (1:400 dilution; Abcam), and Hes1 (1:400 dilution; Abcam), respectively, overnight. Then, the sections were incubated with secondary antibodies (1:20,000 dilution; Abcam), followed by the DAB development and the subsequent hematoxylin staining. Sections were observed under the microscope.

2.8 TUNEL assay

The apoptosis in heart tissue sections was assessed using TUNEL Apoptosis Detection Kit III-FITC (BOSTER, Wuhan, China) according to the instructions. The images were observed under a microscope (Olympus, Tokyo, Japan). The bright yellow-green spots represent the positive results of apoptosis.

2.9 Statistical analysis

Data are expressed as mean value ± SD. The SPSS 25.0 software (SPSS, Chicago, IL, USA) was used for statistical

analysis. The ANOVA followed by Tukey's test was performed for data comparison. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Blocking TLR4 reduces cardiac dysfunction and myocardial injury in LPS-induced sepsis

Cardiac Doppler ultrasound was performed to evaluate the cardiovascular function of rats in each group. Our results showed that, in the rats from the LPS group, the LVEDV and LVESV were significantly higher, while the EF and FS of the rats in the LPS group were significantly lower than the control group and the LPS + TAK242 group (Figure 1). There were no significant differences in LVEDV, LVESV, EF, and FS between the LPS + DAPT group and the LPS group (Figure 1). These results suggest that there is heart dysfunction in the rat model with sepsis. Moreover, blocking the TLR4 pathway with TAK242 could significantly improve heart dysfunction, while this effect was not observed after blocking the Notch pathway with DAPT.

To detect the pathological changes in the heart of rats with sepsis, the H&E staining was performed. Compared with the control group, rats in the LPS group had more obvious myocardial interstitial edema, disordered myocardial fiber arrangement, cardiomyocyte edema, nuclear necrosis, and obvious inflammatory cell infiltration (Figure 2a). Compared with the LPS group, the myocardial fibers in rats from the LPS + TAK242 group were arranged more neatly, the inflammatory cell infiltration was reduced, and the nuclear necrosis was improved. Compared with the LPS group, the LPS + DAPT group had less inflammatory cell infiltration, but the degeneration and necrosis of cardiomyocytes were not improved. These results indicate that sepsis could cause myocardial tissue damage, which could be reduced by TAK242 but not DAPT.

Moreover, TUNEL staining showed that the apoptosis rate of the LPS group was higher than that of the control group (Figure 2b). The apoptosis rate of the LPS + DAPT group was not evidently different from the LPS group. However, the apoptosis rate of the LPS + TAK242 group was lower than that of the LPS group. Western blot analysis showed that the expression levels of the caspase-3 in the LPS group were significantly higher than in the control group and the LPS + TAK242 group (Figure 2c). There was no significant difference in caspase-3 between the LPS group and the LPS + DAPT group, indicating that sepsis may increase the apoptosis of cardiomyocytes. TAK242, but not DAPT, could reduce cardiomyocyte apoptosis caused by sepsis.
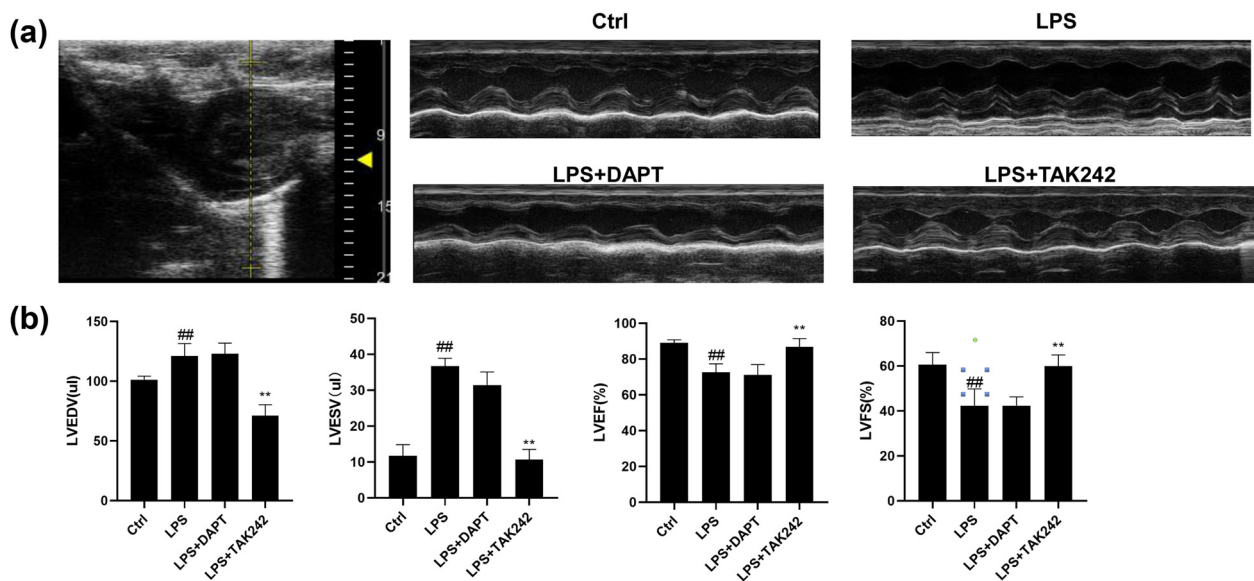

Figure 1: Analysis of heart function. (a) The large picture on the left panel is a representative picture of the position of the heart under echocardiography, located in the left ventricle. The four pictures on the right are representative echocardiography pictures of rats in the control, LPS, LPS + DAPT, and LPS + TAK242 groups. (b) Histogram of LVEDV, LVESV, EF, and FS measured by echocardiography. Compared with control, [#]$P < 0.05$, [##]$P < 0.01$; and compared with LPS group, [*]$P < 0.05$, [**]$P < 0.01$.
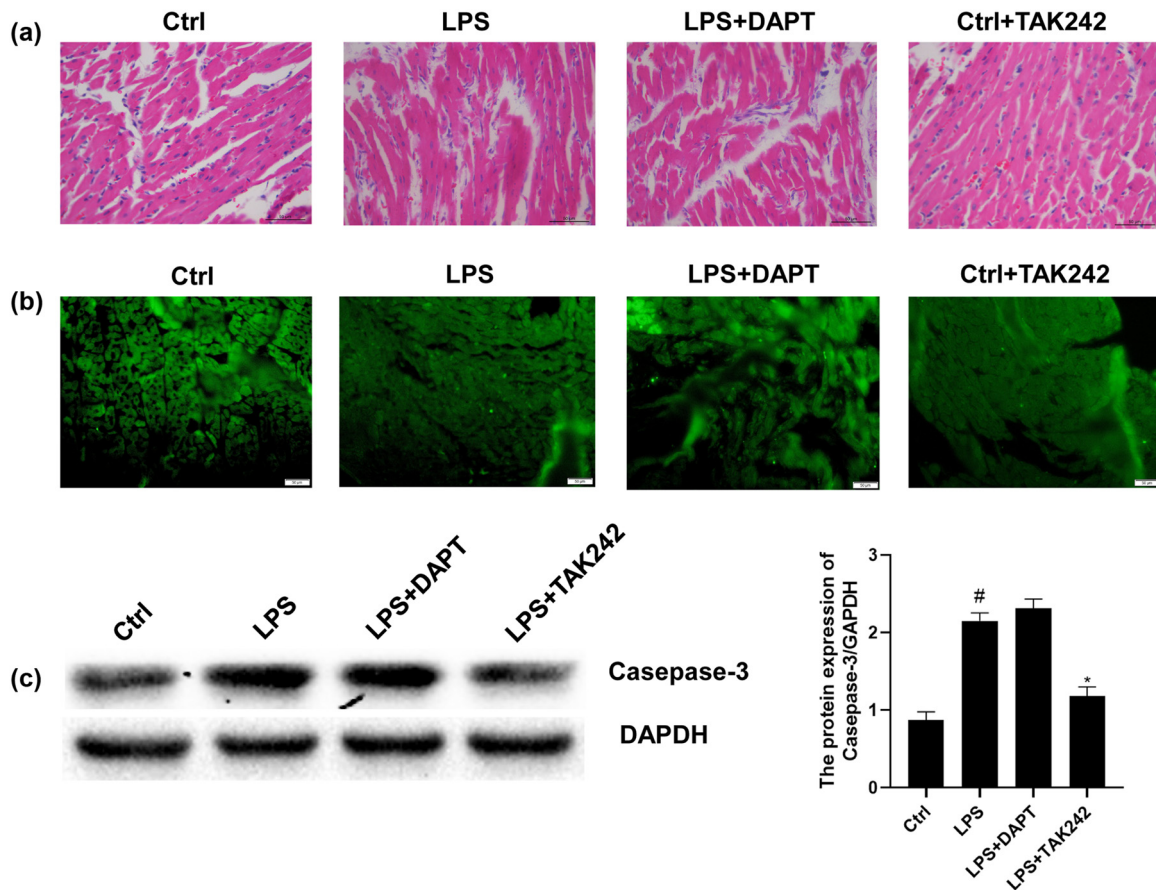
Figure 2: Analysis of histopathological changes and casepase-3. (a) Myocardial H&E staining (×400). (b) TUNEL apoptosis Detection (×400). (c) Expression levels of caspase-3 were detected with the Western blot analysis. Compared with control, $^{#}P < 0.05$; and compared with LPS group, $^{*}P < 0.05$.

Together, blocking the TLR4 pathway by TAK242 may have protective effects on the septic myocardium, while the protective effects were not observed after blocking the Notch pathway with DAPT.

These findings suggest that the TLR4 and Notch signaling pathways may jointly regulate the release of inflammatory cytokines in the septic heart, and the degree of septic myocardial damage may be related to these inflammatory factors.

3.2 Blocking TLR4 or NOTCH reduces inflammatory responses in the heart in LPS-induced sepsis

To detect the levels of local inflammation in the heart tissue, the mRNA and protein expression levels of IL-6 and TNF-α in the heart tissue were assessed. Our results showed that, compared with the control group, the IL-6 and TNF-α mRNA and protein expression levels in the myocardium were significantly increased when the rats were stimulated with LPS (Figure 3a and b). Moreover, compared with the LPS group, the TAK242 and DAPT intervention significantly reduced the mRNA and gene expression levels of IL-6 and TNF-α in the myocardium.

3.3 LPS-induced sepsis activates NOTCH, and blocking TLR4 reduces activated NOTCH

Both immunohistochemistry (Figure 4a) and Western blot (Figure 4b) results showed that the contents of NICD and Hes1 were increased after the LPS stimulation, indicating that the LPS stimulation can activate the Notch signaling pathway in the myocardium. After the DAPT administration, the contents of NICD and Hes1 were decreased, indicating the success of the DAPT intervention. Moreover, compared with the LPS group, TAK242 intervention reduced the protein expression levels of
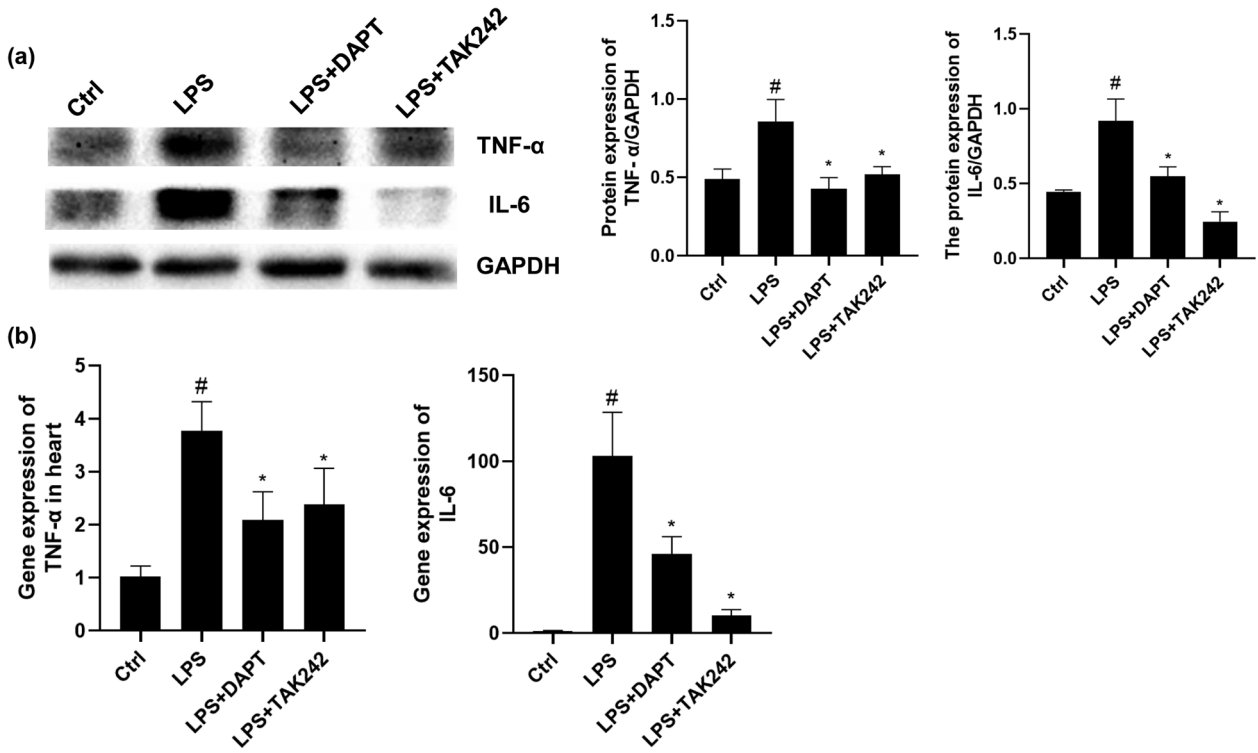
Figure 3: Analysis of cytokine levels. (a) Expression levels of TNF-α and IL-6 were detected with the Western blot analysis. (b) The mRNA expression levels of *TNF-α* and *IL-6* were detected with quantitative real-time PCR. Compared with control, [#]$P < 0.05$; and compared with LPS group, [*]$P < 0.05$.
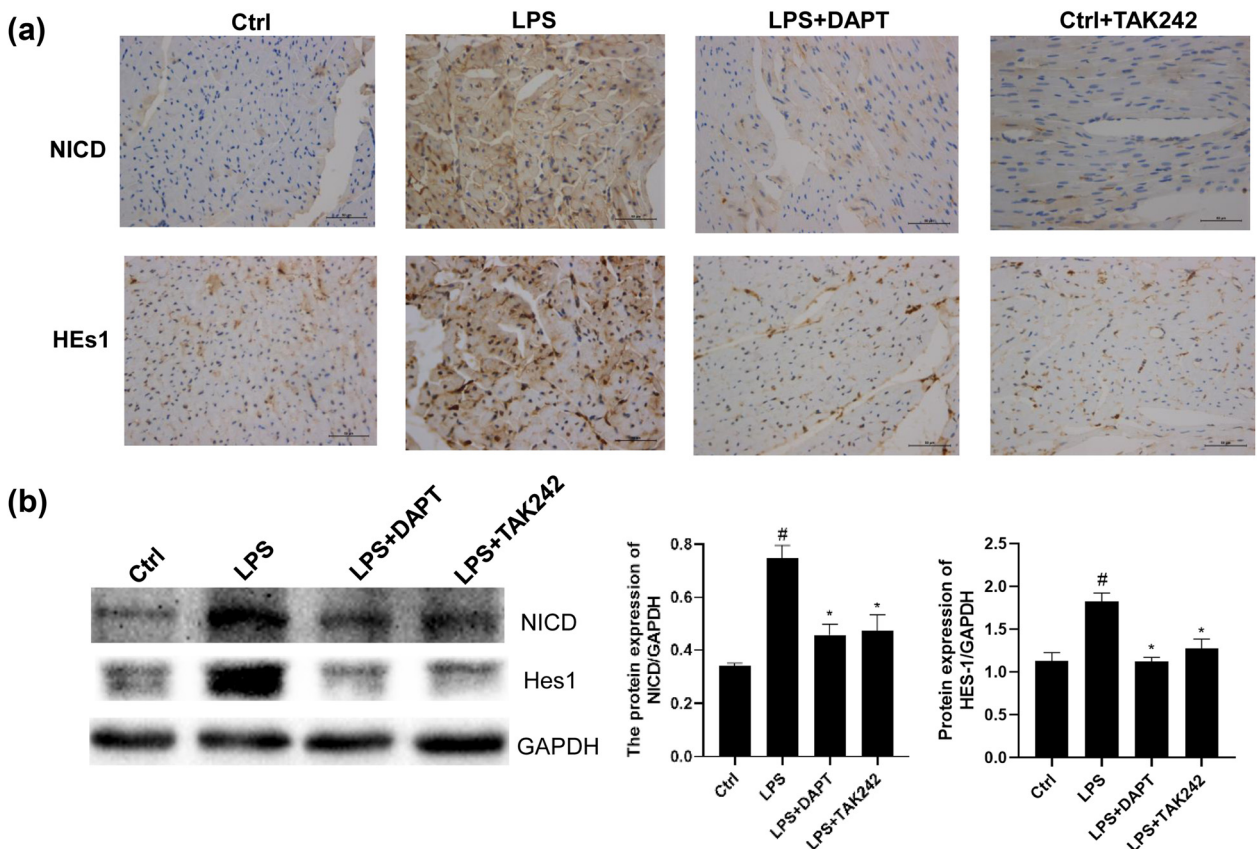

Figure 4: Analysis of NICD and Hes1 proteins in NOTCH signaling pathway. (a) Expressions of NICD and Hes1 were detected with immuno-histochemistry (×400). (b) Expression levels of NICD and Hes1 were detected with the Western blot analysis. Compared with control, [#]$P < 0.05$; and compared with LPS group, [*]$P < 0.05$.

NICD and Hes1, indicating that the activation of Notch signal in septic myocardium is related to the activation of TLR4 signal.

3.4 Blocking TLR4 or NOTCH inhibits NF-κB nuclear translocation and activation in LPS-induced sepsis

Our results by immunohistochemistry (Figure 5a) and Western blot (Figure 5b) demonstrated that the LPS stimulation increased the nuclear translocation of NF-κB and increased the P-NF-κB/NF-κB ratio in the myocardium. Compared with the LPS group, NF-κB nuclear translocation was decreased, and the P-NF-κB/NF-κB ratios were declined, after the TAK242 intervention, indicating that the TLR4 pathway may act through the NF-κB pathway in the septic myocardium. Compared with the LPS group, the NF-κB nuclear translocation was decreased, and

the P-NF-κB/NF-κB ratio was reduced when DAPT was given, indicating that the Notch signaling may increase NF-κB phosphorylation and nuclear translocation to regulate the inflammatory response induced by the TLR4-NF-κB pathway.

4 Discussion

In this study, our results showed that the LPS stimulation could activate the TLR4 and Notch signaling pathways in the heart tissue, and the mutual crosstalk relationship between the TLR4-NF-κB pathway and the Notch pathway could jointly regulate the inflammatory response in the septic heart. The TLR4 pathway activated by LPS could enhance the activation of the Notch pathway, which could further enhance the activation of the TLR4-NF-κB pathway, thereby enhancing the inflammatory response mediated by TLR4. Moreover, the Notch signaling may
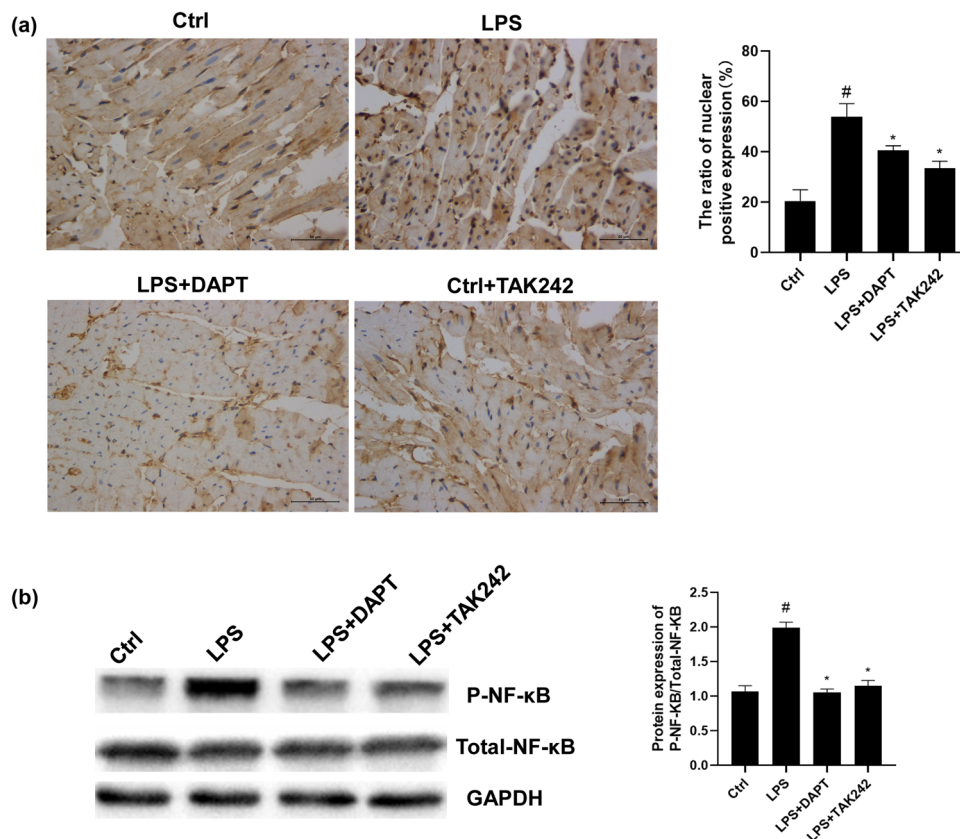

Figure 5: Analysis of NF-κB protein. (a) Nuclear translocation of NF-κB in rat myocardium was detected with the immunohistochemistry. The bar graph represents the ratio of the number of positive nuclei to the total number of nuclei as shown by immunohistochemistry. The larger the ratio, the higher the degree of nuclear translocation (×400). (b) The phosphorylation of NF-κB in rat myocardial tissue was detected with the Western blot analysis. Compared with control, $^{#}P < 0.05$; and compared with LPS group, $^{*}P < 0.05$.

activate NF-κB to enhance the inflammatory responses. Blocking the TLR4 pathway with TAK242 effectively prevented heart dysfunction and myocardial damage in sepsis rats, which was not observed with DAPT.

Studies have shown that the activation of TLR4 signaling in severe sepsis can enhance the inflammatory response by promoting the release of pro-inflammatory cytokines, causing heart damage, and inhibiting heart function [22,24]. Moreover, the TLR4 expressed by cardiomyocytes plays an important role in the acute phase of septic heart dysfunction [25]. On the contrary, the activation of TLR4 can increase the expression of G protein-coupled receptor kinases (GRK2) [37]. GRK2 can lead to the phosphorylation of β2-adrenergic receptors (β2AR), and the phosphorylated β2AR can inhibit the activation of NF-κB through β-arrestins to suppress the inflammatory response [38]. In a mouse model of myocardial infarction with knockdown of β1-adrenergic receptors (β1AR), the inflammatory response was enhanced after GRK2 inhibition. However, β2AR-mediated cardiac contractility was found to be enhanced, β2AR anti-apoptotic signaling was enhanced, and mouse survival was increased [39]. Therefore, the TLR4 signaling pathway can promote the inflammatory response through NF-κB to mediate cardiac injury. Meanwhile, the TLR4 signaling pathway can also increase the expression of GRK2, and GRK2 can inhibit the activation of NF-κB through the β2AR-β-arrestins pathway to reduce the inflammatory response. TAK242 can selectively inhibit the TLR4 signaling pathway and reduce the release of various inflammatory factors in sepsis, thereby reducing the mortality of sepsis [40,41]. TAK-242, a specific inhibitor of TLR4 signaling, can inhibit MyD88 and TRIF-dependent pathways by binding to Cys747 in the intracellular domain of TLR4 [42,43]. Previous studies have shown that TAK-242 prevented acute kidney injury and lung injury in LPS-injected sheep and mice [44,45]. TAK242 can protect against LPS-induced cardiac dysfunction and myocardial injury by blocking the TLR4-mediated inflammatory response [46]. Consistently, in this study, after using TAK242 to block the TLR4 signaling pathway in septic rats, the activation and nuclear translocation of NF-κB were reduced, the contents of inflammatory factors (TNF-α and IL-6) were reduced, and the heart damage was improved, indicating that the activation of the TLR4 signaling pathway in sepsis could promote the inflammatory response through the NF-kB pathway, and lead to myocardial damage and heart dysfunction.

In terms of the TLR-mediated regulation of Notch signaling, TLRs may indirectly regulate the Notch signaling by inducing the expression of Notch receptors and ligands, thereby activating the Notch signaling pathway. Many studies have reported increased expression levels of Notch receptors and ligands after activation of TLRs [29–31,47]. A previous study indicated that TLR4 signaling pathway activation resulted in an elevation of DLL4 expression through ERK/FOXC2 signaling pathway [48]. Another study showed that TLR4–NF-κB signaling induced hepatocyte Jag1 expression and triggered inter-hepatocyte Jagged1/Notch signaling [49]. The Notch signaling pathway regulates the differentiation, proliferation, survival, and development of cells [50]. In addition, the Notch signaling regulates the production of cytokines in T lymphocytes and macrophages [29,31]. However, it is not clear whether Notch is involved in the enhancement of inflammatory response and myocardial damage in septic heart tissue. Our results showed that the content of NICD was increased after LPS activated the TLR4 pathway. When TAK242 was used to block the TLR4 pathway, the content of NICD was decreased, indicating that the Notch activation in the septic heart may be related to the activation of TLR4 signal.

It is shown that the activation of Notch signals can promote the differentiation of most immune cells to a pro-inflammatory phenotype, thereby increasing the inflammatory responses [51,52]. In the rat myocardial infarction model, the inhibition of Notch signal could reduce the differentiation of macrophages into M1 macrophages, thereby reducing the levels of inflammatory factors and reducing the inflammatory responses [33]. These findings have shown that Notch signaling can promote inflammatory responses. DAPT is often used as a specific inhibitor of γ-secretase and a blocking agent of Notch pathway [53]. DAPT inhibits the formation of the soluble NICD protein by preventing the cleavage of γ-secretase at the S3 site of the Notch receptor [54,55]. In this study, to verify the role of the Notch1 signaling pathway in the inflammatory response induced by TLR4 in the heart, the γ-secretase inhibitor DAPT was used to inhibit the activation of Notch in the septic rats. After DAPT intervention, the inflammatory factors (TNF-α and IL-6) were decreased, indicating that the inhibition of Notch signal would reduce the inflammatory response of the heart tissue. Therefore, the activation of Notch signal in sepsis may play an important role in the enhancement of the inflammatory response induced by TLR4 in the heart.

Our results showed that the Notch signaling had an important effect on the activation of NF-κB in the heart. The DAPT was used to inhibit the Notch signaling pathway during LPS stimulation, which attenuated the phosphorylation and nuclear translocation of NF-κB p65. Studies have shown that the Notch1 signaling pathway in

macrophages can interact with NF-κB to enhance the inflammatory responses induced by TLR4 [56,57]. Moreover, in the interstitial cells of human aortic valve stenosis, Notch1 enhances the inflammatory response and promotes the osteogenic response under the stimulation of TLR4 by regulating the activation of NF-κB [58,59]. NOTCH3 is the only NOTCH receptor expressed in resting macrophages [60]. In macrophages isolated from patients with atherosclerosis, NOTCH1 appears to play a more prominent role than those of NOTCH2 and NOTCH3 in the regulation of the NF-κB signaling pathway and in the induction of pro-inflammatory gene expression [61]. On the contrary, NOTCH4 exhibits anti-inflammatory activity in activated macrophages by interfering with interferon-gamma, TLR4 signaling, and NF-kB transcriptional activity [62]. Notch signaling can trigger free NF-κB to translocate into the nucleus and stimulate the NF- κB pathway, leading to the activation of pro-inflammatory cytokine genes and causing inflammatory

cytokine secretion [48,63]. After NF-κB is activated, the p50-p65 forms a heterodimer and undergoes nuclear translocation, which triggers the transcription of mRNA encoding a series of mediators, such as adhesion molecules, cytokines, chemokines, and procoagulants [64]. These findings suggest that the Notch signaling pathway plays an important role in NF-κB-mediated inflammation.

In this study, however, when DAPT was used to block the Notch pathway, the septic heart dysfunction and myocardial damage showed no obvious improvement. The possible reason would be that Notch could mediate inflammation and may also have repairing effects. Previous findings indicated that the activation of Notch signaling pathway played an important role in the regeneration of endocardium injury, which may play a complex role in the interaction with different signaling molecules [65]. Blocking the Notch pathway in the infarct model could reduce the infiltration of inflammatory cells and the inflammatory responses in the heart, which,
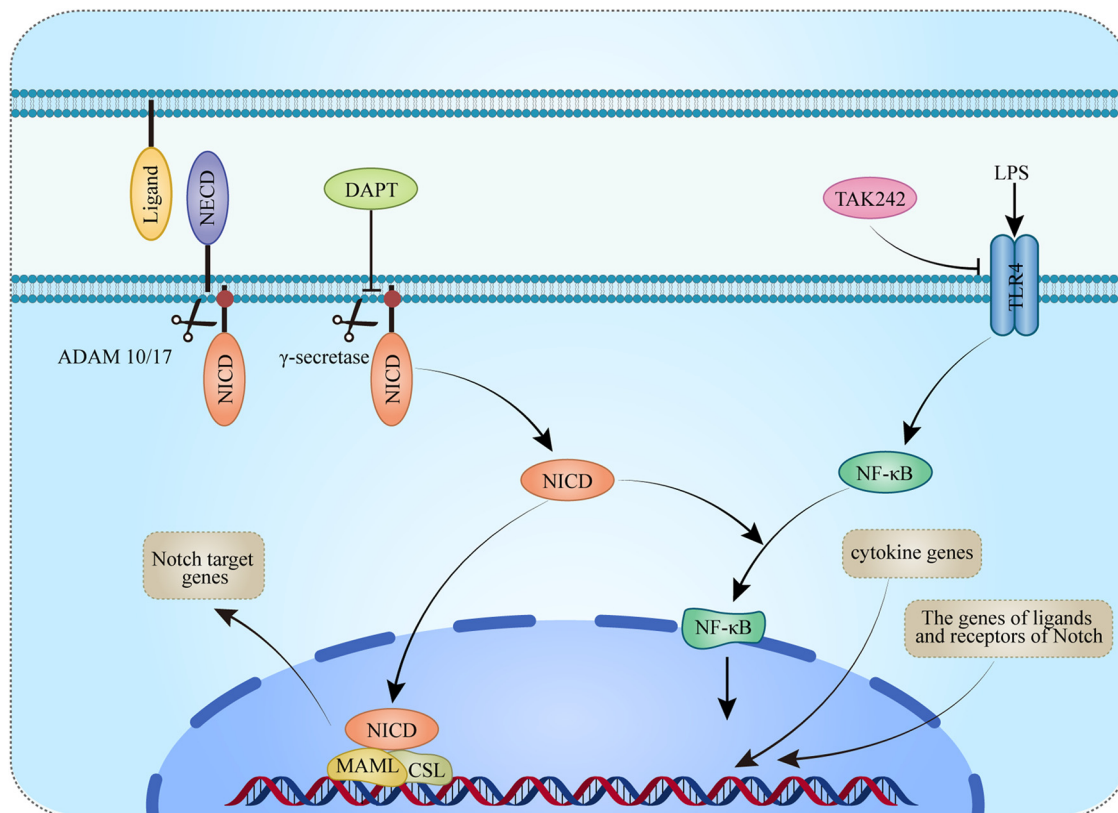

Figure 6: A schematic figure illustrating the interaction between TLR4 and Notch1 signaling pathways. The TLR4 receptor activated by LPS promotes the expression of inflammatory factors and the expression of Notch receptors and ligands through the NF-KB pathway. The increase in the expression of Notch receptors and ligands promotes the activation of the Notch signaling pathway. After activation, the Notch intra-cellular domain is released into the cytoplasm, which enhances the inflammatory response by promoting the activation of NF-KB. This study used TAK242 to block the TLR4 pathway and DAPT to block the Notch pathway. The relationship between the two pathways was investigated.

however, did not reduce the area of the heart infarction but increased the infarction area [33]. On the other hand, Notch is crucial in promoting the survival of cardiomyocytes and endothelial cells and maintaining the contractile phenotype of VSMCs [66]. In the cardiovascular system, Notch activation prevents apoptosis of cardiomyocytes [67] and endothelial cells caused by different types of insults [68]. Thus, activation of Notch in the heart [69] and endothelium [70] could represent a new therapeutic approach against diseases such as coronary artery disease and heart failure [71]. It has been demonstrated in mouse models that Notch signals over-expressed in vascular, stromal cells could significantly improve the repairing ability of mesenchymal stem cells [72]. Notch signal plays an important role in the repairing of vascular endothelial injury [73]. Notch1 and Jagged1, expressed in the adult heart, can protect cardiac tissue under pathophysiological conditions [72]. Notch1 signaling is activated following myocardial injury by repressing reactive oxygen species production and by stabilizing mitochondrial membrane potential [74]. Notch1 signaling can attenuate myocardial ischemia/reperfusion injury by suppressing oxidative/nitrative stress [75]. These findings suggest the damage repairing effects of the Notch signal. However, the repairing mechanism of Notch in septic heart injuries needs further verification.

5 Conclusion

In conclusion, our results showed the interaction between TLR4 and Notch signaling pathways may enhance the inflammatory response in septic rat hearts by regulating the activation of NF-κB (Figure 6). Blocking the TLR4 pathway with TAK242 could improve heart dysfunction and myocardial damage in sepsis. Blocking the Notch pathway with DAPT could not effectively prevent heart dysfunction and myocardial damage in rats with sepsis. The possible reason is that Notch can mediate inflammation and may also have repairing effects. Further studies are warranted for verification.

Author contribution: Study design: Ziyang Liu and Peng Peng; data collection: Ziyang Liu, Wenli Li, Yang Cao, Xiaoxia Zhang, Kai Yang, and Meng Yang; statistical analysis: Xiaoxia Zhang, Kai Yang, and Fukang Yin; data interpretation: Ziyang Liu; manuscript preparation: Ziyang Liu and Peng Peng; literature search: Wenli Li and Yang Cao; and funds collection: Peng Peng.

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