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Original Research Article

Effect of Baicalein Inhibiting miR-155 through STAT3 Signaling Pathway on Inflammatory Response in Mice with Acute Appendicitis

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Abstract

Background: Acute appendicitis is caused by infection and inflammation of the appendix. Baicalein (ABC) can reduce inflammation through various mechanisms, but its water solubility is poor. Liposomes have good biocompatibility and drug delivery ability.

Objectives: This study aims to explore the therapeutic effect of baicalein liposome nanoparticles (LNS-ABC) with a good targeting effect on acute appendicitis and provide a basis for developing new drugs.

Materials and Methods: The LNS-ABC complex was prepared, and a mouse model of acute appendicitis was constructed. The levels of inflammatory factors in mice were measured. The relationship between miR-155 and signal transducer and activator of transcription 3 (STAT3) was investigated by luciferase assay. Using miR-155 agonists and inhibitors and STAT3 agonists and inhibitors, the mechanism of action of LNS-ABC in mice with acute appendicitis was investigated.

Results: ABC can effectively inhibit the inflammatory response in acute appendicitis, and the inhibitory effect of LNS-ABC is more effective. Moreover, LNS-ABC inhibits the expression of miR-155 by regulating and inhibiting the STAT3 signaling pathway, thereby improving inflammation in acute appendicitis.

Conclusion: Baicalein can effectively inhibit the inflammatory response of acute appendicitis mice, and LNS-ABC has a better curative effect. LNS-ABC can inhibit the expression of miR-155 by inhibiting the STAT3 signaling pathway, thus improving the inflammatory response of acute appendicitis. LNS-ABC is an essential strategy for the treatment of acute appendicitis and its inflammatory reaction.

Keywords

Baicalein, signal transducer and activator of transcription 3 signaling pathway, miR-155, acute appendicitis

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Introduction

Acute appendicitis is an infection and inflammation of the cecal diverticula (appendix) and is a common acute abdominal pain (Coccolini et al., 2018). The symptoms of acute appendicitis are often accompanied by an inflammatory response, so there is a need to control inflammation (Sener et al., 2023). At present, the main treatment methods for acute appendicitis are surgery and medical control. However, surgical treatment has certain risks, and the toxic side effects of drugs cannot be ignored. Therefore, it is very important to find new therapeutic approaches.

Studies have shown that baicalein has anti-inflammatory effects and inhibits the activity of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and reduces its nuclear translocation and deoxyribonucleic acid (DNA) binding capabilities, thereby downregulating the expression of NOX genes, reducing the generation of reactive oxygen species (ROS), and reducing oxidative stress (Ji et al., 2019). Baicalein can also significantly reduce the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), inhibit the phosphorylation of NF-κB and signal transducer and activator of transcription 1 (STAT1), and the

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production of NOX2, reduce the expression of pro-inflammatory factors such as tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin 6 (IL-6), and inhibit the lipopolysaccharide (LPS)-induced inflammatory response of BV-2 cells to the greatest extent (Kim et al., 2023). However, the poor water solubility, low bioavailability, and insufficient *in vivo* stability of baicalein limit its clinical application. To solve these problems, liposome nanoparticles (LNS), as an efficient drug delivery system, have been widely used to improve the solubility, stability, and targeting of drugs (Fu et al., 2021). Studies have shown that baicalein liposome nanoparticles (LNS-ABC) can significantly improve the bioavailability of the drug and enhance its anti-inflammatory effect.

Wogonin inhibits the production of ROS in macrophages, reduces the nuclear localization of NF-κB to a certain extent, and weakens the inflammatory response of IL-1β, TNF-α, and other interferences. Baicalein can significantly reduce iNOS and COX-2 to a certain extent, inhibit NF-κB, STAT1 phosphorylation, and NOX2 production, and inhibit LPS-induced BV-2 cell inflammation to the greatest extent. Although baicalein has significant anti-inflammatory effects, there are few reports on its role in inflammation in acute appendicitis, and further research is needed.

Signal transducer and activator of transcription 3 (STAT3) is an important factor in maintaining cell proliferation and survival and can be activated by various cytokines to mediate immune and inflammatory responses in response to injury (Wang et al., 2022). In the intestinal tissues of inflammatory bowel disease (IBD), STAT3 is abnormally activated by a variety of pro-inflammatory cytokines such as IL-6, IL-23, and TNF-α, which destroy the integrity of the intestinal barrier and over activates the innate immunity and Th17 cell-mediated immune response, resulting in long-term inflammation of the intestine. Eventually, intestinal fibrosis, intestinal stenosis, and other complications occur (Zhang et al., 2020). However, the specific mechanism of action of STAT3 in acute appendicitis is still unclear and needs further study.

MiR-155 is upregulated in colorectal cancer (CRC) tissues and related to the occurrence and development of CRC (Amerikanou et al., 2021). Inhibition of miR-155 may be a new therapeutic strategy for CRC. In addition, miR-155 can regulate the composition and diversity of intestinal microbiota and inhibit the expression of anti-inflammatory genes in intestinal epithelial cells, thus promoting the occurrence and development of intestinal inflammation. Studies have reported that miR-155 activates the IL-1 signaling pathway and promotes the upregulation of pro-inflammatory factors by targeting the TAB1 protein in intestinal macrophages. This will cause Th17 cells to produce an immune response, manifested by increased lamina propria mononuclear cells (LPMC) (Kim et al., 2022). Moreover, the frequency of CD8+IL-17+Th17 cells was increased, thereby aggravating intestinal inflammation. In terms of participating in intestinal immune regulation, miR-155 promotes Treg cells to secrete IL-10 and inhibits immune inflammation in appendicitis (Xie et al., 2014). However, there are few studies on the role of miR-155 in acute appendicitis and its relationship with the STAT3 signaling pathway, and it is not clear whether baicalin plays an anti-inflammatory role by regulating the STAT3/miR-155 pathway.

Although baicalein has significant anti-inflammatory potential, there are few studies on its application in acute appendicitis, and the problems of its water solubility and bioavailability have not been effectively solved. In addition, there is no clear conclusion on whether baicalein plays an anti-inflammatory role by regulating the STAT3/miR-155 pathway. Based on this, this study aimed to investigate the effect of LNS-ABC on the inflammatory response of acute appendicitis and its mechanism of action. By constructing a mouse model of acute appendicitis, this study will systematically evaluate the regulatory effects of LNS-ABC on inflammatory factors, the STAT3 signaling pathway, and miR-155, providing a new theoretical basis and potential therapeutic strategy for treating acute appendicitis.

Materials and Methods

Instruments, Reagents, and Animals

Baicalein (purity ≥98%, catalog number: B3010, Beijing ETA Bio); liposome nanoparticles (catalog number: LD-1001, Dalian Lidi Fluid Control); miR-155 antibody (catalog number: KM-155, Shanghai Kemin Bio); TNF-α kit (catalog number: TNF-001, Beijing ETA Bio); IL-1β, IL-6 kit (catalog number: IL-1002, Shanghai Jianglai Biotechnology).

Animals

Twenty-four male Sprague–Dawley (SD) rats, aged 8–10 weeks and weighing 200–250 g (Beijing Vitong Lever). All animal experiments are conducted by the International Guidelines for the Care and Use of Laboratory Animals and are approved by the Laboratory Animal Ethics Committee (IAEC) (approval number: IAEC-2023-001, date of approval: January 15, 2023). Animals are kept at a constant temperature (22°C ± 2°C), constant humidity (50% ± 10%), and a 12-h light/dark cycle.

Method

Preparation of LNS-ABC Nanoparticles

Baicalein solution with a 10 mg/mL concentration was prepared by dissolving baicalein in dichloromethane. Mix phospholipids and Chol in a ratio of 1:5. Baicalein solution was slowly dropped into the liposome mixture at a rate of 1 mL/min. It was thoroughly mixed using a magnetic stirrer (model: MS-300, stirring speed: 500 rpm, temperature: 25°C) or an ultrasonic processor (model: UP-200S, voltage: 200 V, duty

cycle: 50%). Ultrasonicate for 30 min to obtain a homogeneous mixed solution. A rotary evaporator (model: RE-2000) was used to remove the organic solvent and obtain a liposome nanosuspension. The LNS-ABC suspension was sterilized by filtration through a 0.22 μm filter membrane. The suspension of LNS-ABC was diluted to 1 mg/mL with ultrapure water, and its morphology was observed by transmission electron microscope (model: JEM-1400, acceleration voltage: 80 kV).

The mean particle size and particle size distribution of the LNS-ABC nanoparticles were measured using a dynamic light scattering (DLS, model: Zetasizer Nano ZS90). The entrapment rate of baicalein was determined by the ultrafiltration centrifugal method. The LNS-ABC suspension was placed in an ultrafiltration centrifuge tube (molecular weight retention: 10 kDa) and centrifuged at 10,000 rpm for 15 min to collect the filtrate. The concentration of free baicalein in the filtrate was determined by high-performance liquid chromatography (HPLC) (model: Agilent 1260).

Mouse Modeling of Acute Appendicitis

Forty mice were selected, five of which were used as a control group without any treatment. Anesthesia was administered with isoflurane (isoflurane, catalog number: ISO-1001) at a dose of 3% to induce anesthesia and 1.5% to maintain anesthesia, administered by inhalation through a mask (Sag et al., 2022). An incision of about 1 cm was made in the right lower abdomen of the mice to expose the appendix. Ligate at the base of the appendix using no. 4 thread (catalog number: SUT-4001), ensuring the ligature is firm but does not damage the surrounding tissue. The abdominal incision was closed with absorbable sutures (catalog number ABS-2001), and an anti-biotic ointment (catalog number ANT-3001) was applied postoperatively to prevent infection. After surgery, the mice were placed in a warm (28°C) and quiet environment to recover. They were provided with adequate water and food, and observed daily for 5 days for their activity status, incision healing, and weight change. When the mice showed apparent local congestion, edema, and inflammatory cell infiltration, and the serum white blood cell count and C-reactive protein (CRP) level were significantly increased, the model was successfully constructed. The mice that successfully built the model were divided into seven groups of five mice each. They were respectively the NC group, baicalein (ABC) group, LNS-ABC group (baicalein liposome nanoparticle group), LNS-ABC+miR-155 agonist group, LNS-ABC+ miR-155 inhibitor group, LNS-ABC+STAT3 agonist group, and LNS-ABC+STAT3 inhibitor group.

Cure Criteria

Model mice without any treatment generally die within 1–2 days. Baicalein treatment usually returns to normal in about 10 days, and surgery or LNS-ABC treatment usually returns to normal in about a week. Mice that survived for 5 days with significant improvements in serum CRP levels and histopathological scores were considered cured.

Hematoxylin and Eosin (HE) Staining

The tissue was fixed in 10% formalin, embedded, and cut into 5 μm cross-sections at 20°C. The slides were attached to the tissue surface, dried overnight at 4°C, and finally frozen at 20°C. The prepared transverse sections were stained for pathological ossification using HE. Images were then collected using an optical microscope (Nikon ECLIPSE Ti-S, Ruike Zhongyi Co., Ltd.).

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) Detection

Ribonucleic acid (RNA) was extracted using TRIzol reagent (catalog number: TRI-1001) and reverse-transcribed into cDNA using a reverse transcription kit (catalog number: RT-2001, kit, Shanghai Caiyou Industrial). qRT-PCR was performed using SYBR Green Master Mix (catalog number: SYB-3001) under the condition of predenaturation at 95°C for 5 min, followed by 40 cycles (denaturation at 95°C for 15 s, annealing/extension at 60°C for 30 s). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is an intrinsic control between ROCK/MLCK and miRNA, and the qRT-PCR results were analyzed. Relative levels were estimated using the $2^{-\Delta\Delta Ct}$ method. Table 1 lists the primers and primer sequences.

Western Blot

Total protein was extracted using radioimmunoprecipitation assay (RIPA) lysis buffer (catalog number: RIPA-1001), and protein concentration was determined using bicinchoninic acid (BCA) protein quantification kit (catalog number: BCA-2001). Using 10% SDS-PAGE protein isolate, after transmembrane with primary antibody (STAT3 antibody, dilution: 1:1,000; GAPDH antibody, dilution: 1:500) incubated overnight. After washing, it was incubated with horseradish peroxidase (HRP)-labeled secondary antibodies (catalog number: HRP-3001, dilution: 1:5,000), for 1 h and then tested with an ECL color developing kit (catalog number: ECL-4001). Detection was enhanced by the internal reference GAPDH.

Statistical Methods

Data analysis was performed using SPSS 21.0 (IBM) and GraphPad Prism 9.0 (GraphPad Software). Data were expressed as mean ± standard deviation, and a *t*-test or one-way analysis of variance (ANOVA) was used for inter-group comparison, with $p < .05$ indicating statistically significant differences.

Table 1. Real-time Polymerase Chain Reaction (PCR) Primers and Primer Sequences.

Primers		Sequences	Units
miR-155a	Forward primer	5-GAGCTCTGGAAGATTAGCATTGTAAACTA-3	nM
	Reverse primer	5-ACCGTTCAAAGTGCTTATTTTTGACA-3	nM
STAT3	Forward primer	5′-GCCCATCTACAGGAAGACC-3′	nM
	Reverse primer	5′-GGTCCAGGAAGCAGGTATTC-3′	nM
GAPDH	Forward primer	5′-AAAGGGTCATCATCTCCGCC-3′	nM
	Reverse primer	5′-AGTGATGGCATGGACTGTGG-3′	nM
JAK	Forward primer	5′-ATGAGGCACAGATACTACTTG-3′	nM
	Reverse primer	5′-TTATGTTTCCCTCCAGCCTC-3′	nM

Note: GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; JAK: Janus kinases; STAT3: Signal transducer and activator of transcription 3.

Results

Successfully Constructed LNS-ABC Nanocomplex and Appendicitis Mouse Model

Through transmission electron microscopy, it was observed that the nanoparticles were spherical, with a complete bilayer, and the surface was smooth and flat, and no obvious drug molecular particles were found, indicating that ABC was successfully encapsulated in the liposomes (Figure 1A). In addition, the DLS measurement results showed that the average particle size of LNS-ABC was 35 nm and the polydispersity index (PDI) was 0.15 ± 0.02, indicating uniform particle distribution (Figure 1B). The potential distribution of LNS-ABC was −12 mV, which was considered stable and conducive to the dispersion and stability of nanoparticles (Figure 1C). The results of high-performance liquid chromatography showed that the encapsulation rate of LNS-ABC was 85% ± 3%. The LNS-ABC material is successfully prepared.

The intestinal mucosa of the model mice had scattered and multiple epithelial necroses, more glands were shed, and inflammatory cells were infiltrated. Compared with normal mice (sham operation group), they showed typical mucosal thickening, edema, and lymphoid follicle hyperplasia (Figure 2A). The mucosal thickness of model mice was (120 ± 10) μm, the mucosal edema score was (3.2 ± 0.3) points, and the number of lymphatic follicles per field was (15 ± 2), which were significantly increased compared with the normal group ($p < .01$, Figure 2B–2D). The results indicated that the acute appendicitis mouse model was successfully constructed.

LNS-ABC Can Improve Inflammation in Acute Appendicitis and Reduce the Gene Expression of STAT3

In order to explore the role of ABC and LNS-ABC in the inflammation of acute appendicitis, we found through animal experiments that the cure rates of mice under the intervention of ABC and LNS-ABC were 60% and 80%, respectively, which are as effective as appendectomy surgery (Table 2); while the levels of inflammatory factors in mice with acute appendicitis (NC group) were higher than healthy mice (control group) (Figure 3A–3C). Under ABC conditions, a downward trend was clearly observed under the intervention of TNF-α, IL-1β, and so on ($p < .05$), and it was further found that the decrease was most significant in the LNS-ABC group (Figure 3A–3C), and in terms of STAT3 expression, the NC group had abnormally strong expression (vs NC group, $p < .05$), while the LNS-ABC group had the lowest expression (vs NC group, $p < .05$, Figure 3D). It shows that LNS-ABC can improve inflammation in acute appendicitis and affect the gene expression of STAT3.

STAT3 Signaling Involves in LNS-ABC's Role in Improving Inflammatory Response in Mice with Acute Appendicitis, and This Process has a Certain Relationship with miR-155

As shown in Figure 4A, we used STAT3 agonists and inhibitors. Corresponding changes in STAT3 were found, and under the intervention of LNS-ABC+STAT3 agonist, the levels of inflammatory factors in mice increased significantly (Figure 4B–4D). On the contrary, the levels of inflammatory factors in the LNS-ABC+S3I-201 group were the lowest state, indicating that LNS-ABC can inhibit the STAT3 signaling pathway to improve inflammation in appendicitis. Through Figure 4E, it is found that LNS-ABC has a positive inhibitory effect on miR-155.

Luciferase Experiment

Luciferase experiment results showed (Figure 5) that compared with the NC group, luciferase activity in the STAT3 wild-type group was significantly increased ($p < .05$), while luciferase activity in the miR-155 inhibitor +STAT3 wild-type group was significantly decreased ($p < .05$). There was no significant change in luciferase activity in the miR-155 inhibitor +STAT3 mutant group. These results suggest that

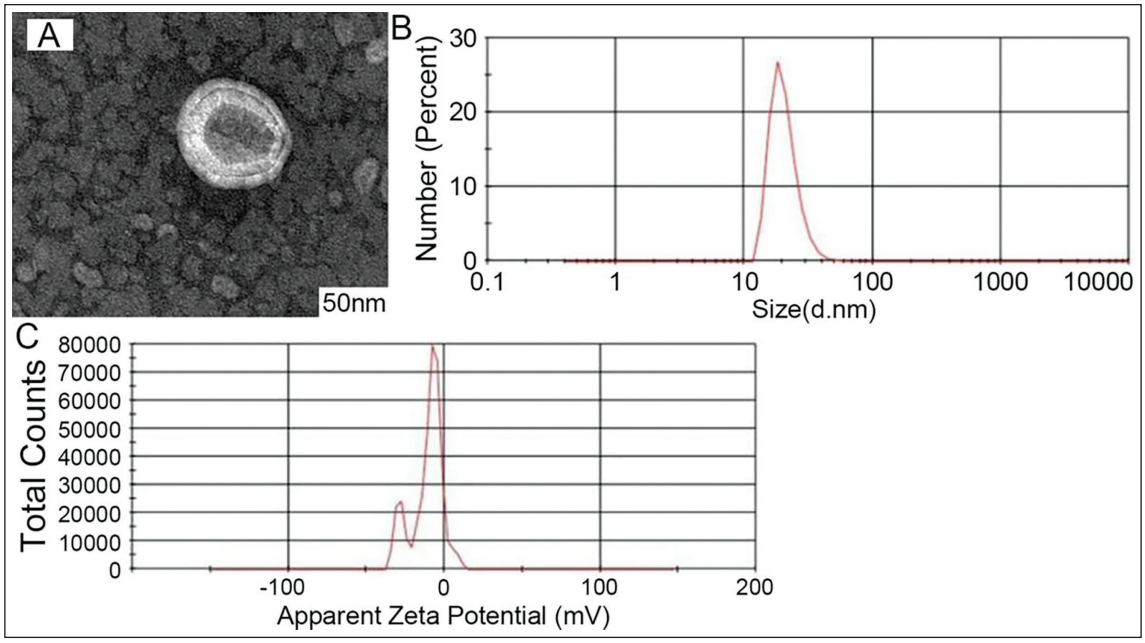


Figure 1. Transmission Electron Microscopy Results of Baicalein Liposome Nanoparticles (LNS-ABC).

Note: (A) Transmission electron microscope image of LNS-ABC; (B) particle size distribution of LNS-ABC; (C) potential distribution of LNS-ABC.

miR-155 regulates the expression of STAT3 by targeting the 3′UTR region.

LNS-ABC Improves Inflammation in Acute Appendicitis and Achieves This by Inhibiting miR-155

We added miR-155 agonist and miR-155 inhibitor, respectively, on the basis of LNS-ABC. The expression levels of related inflammatory factors in the LNS-ABC+miR-155 inhibitor group were similar to the trend of the above-mentioned LNS-ABC+STAT3 inhibitor group, while the LNS-ABC+miR-155 agonist group showed opposite results (Figure 6A–6C). It shows that LNS-ABC improves inflammation in acute appendicitis by regulating the STAT3 signaling pathway and thereby inhibiting the expression of miR-155.

LNS-ABC Inhibits miR-155 Through the STAT3 Signaling Pathway, Thereby Improving the Inflammatory Response in Mice with Acute Appendicitis

After further use of the miR-155 inhibitor, levels of IL-6 and others were reduced (Figure 7A–7C, 7E). In addition, in order to clarify the effect of LNS-ABC on the consumption of miR-155 and STAT3, we now added LNS-ABC+STAT3 inhibitor+miR-155 inhibitor. In contrast to the LNS-ABC+STAT3 agonist+miR-155 agonist group, the level of miR-135 was greatly increased (Figure 7D), and after blocking STAT3, its level also increased, was inhibited, and the expression of inflammatory factors also showed an inhibitory trend (Figure 7E). It shows that LNS-ABC inhibits miR-155 through the STAT3 signaling pathway.

Discussion

Although baicalein has a positive effect in anti-inflammation, as a natural product, its solubility and stability are low, and it is easily affected by factors such as light, heat, and oxidation, which limit its dissolution and absorption in the body (Shen et al., 2019). Therefore, this study uses liposome nanoparticles as carrier materials to encapsulate baicalein in nanoparticles, which can effectively protect baicalein from the influence of the external environment, improve its stability, and extend the shelf life of the drug. Based on the above reasons, we successfully prepared the LNS-ABC complex. To prove that LNS-ABC can improve inflammation in acute appendicitis, we found through animal experiments that inflammatory factors in mice with acute appendicitis were higher than those in healthy mice. Under the intervention of ABC, TNF-α, IL-1β, and IL-6 were significantly reduced, with the most significant reduction in the LNS-ABC group. It shows that LNS-ABC can inhibit inflammation in acute appendicitis. This is because baicalein can inhibit the activation of IKK, thereby blocking IκB degradation, reducing binding of NF-κB to DNA, and inhibiting expression of its downstream inflammation-related genes such as IL-1β,

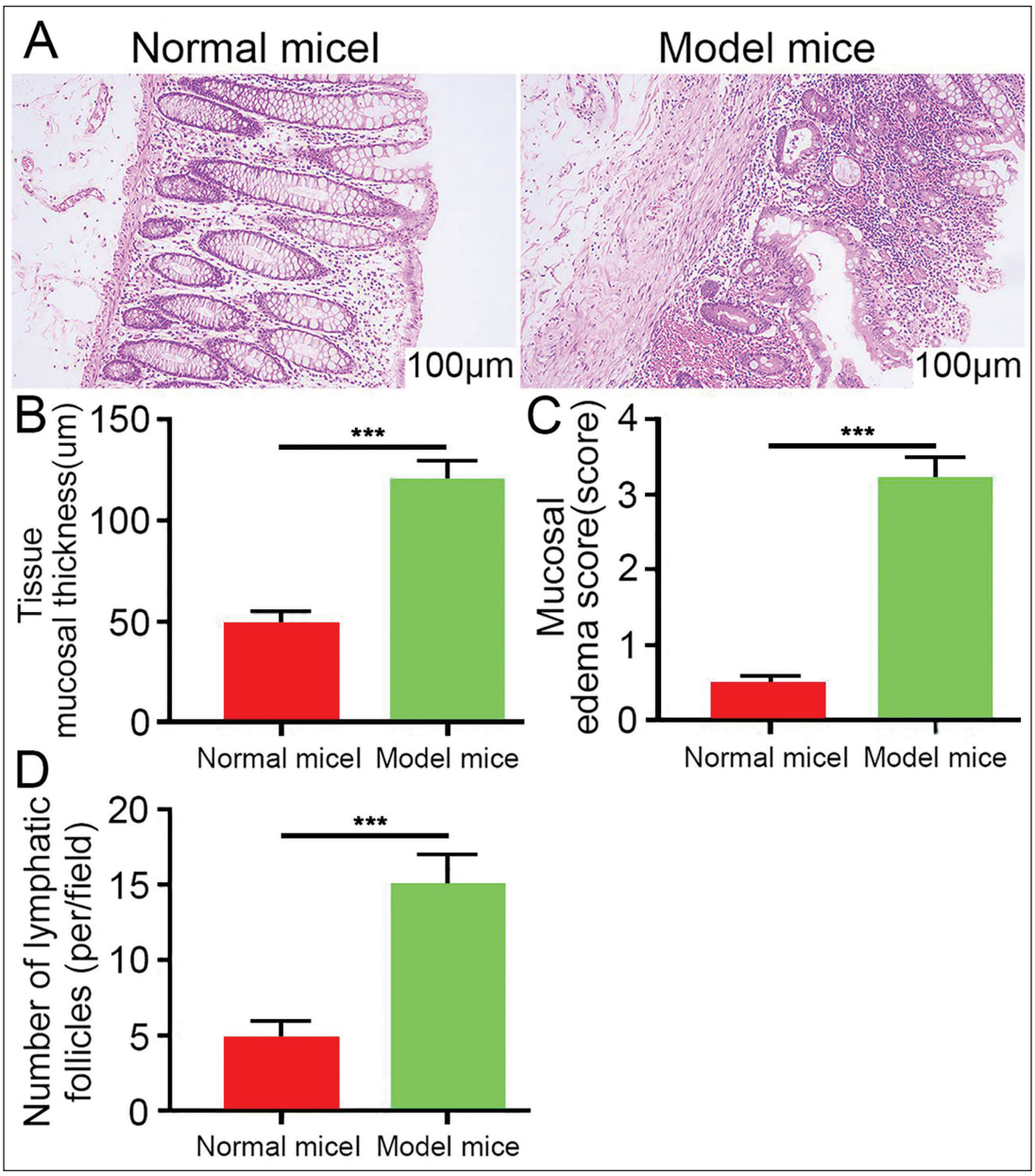


Figure 2. Results of Acute Appendicitis Mouse Model Construction.

Notes: (A) Hematoxylin and eosin (HE) staining results in mice, ×200. Green arrows indicate normal intestinal mucosal structure, and red arrows indicate significant pathological changes, including mucosal thickening, inflammatory cell infiltration, and lymphoid follicular hyperplasia; (B) mucosal thickness of mouse appendices; (C) evaluation of mucosal edema in mice; (D) number of lymphoid follicles in mouse appendix. The error line represents the standard deviation (SD) for each set of data. All experiments were repeated three times. ***$p < .05$.

Table 2. Cure Rate.

Treatment Method	Number	Number of Experiments	Number of Deaths	Cure Rate (%)	*p*
Appendectomy	5	5	0	100	<.01
LNS	5	0	5	0	–
ABC	5	3	2	60	<.05
LNS-ABC	5	4	1	80	<.01
Control	5	0	5	0	–

Note: ABC: Baicalein; LNS-ABC: Baicalein liposome nanoparticles.

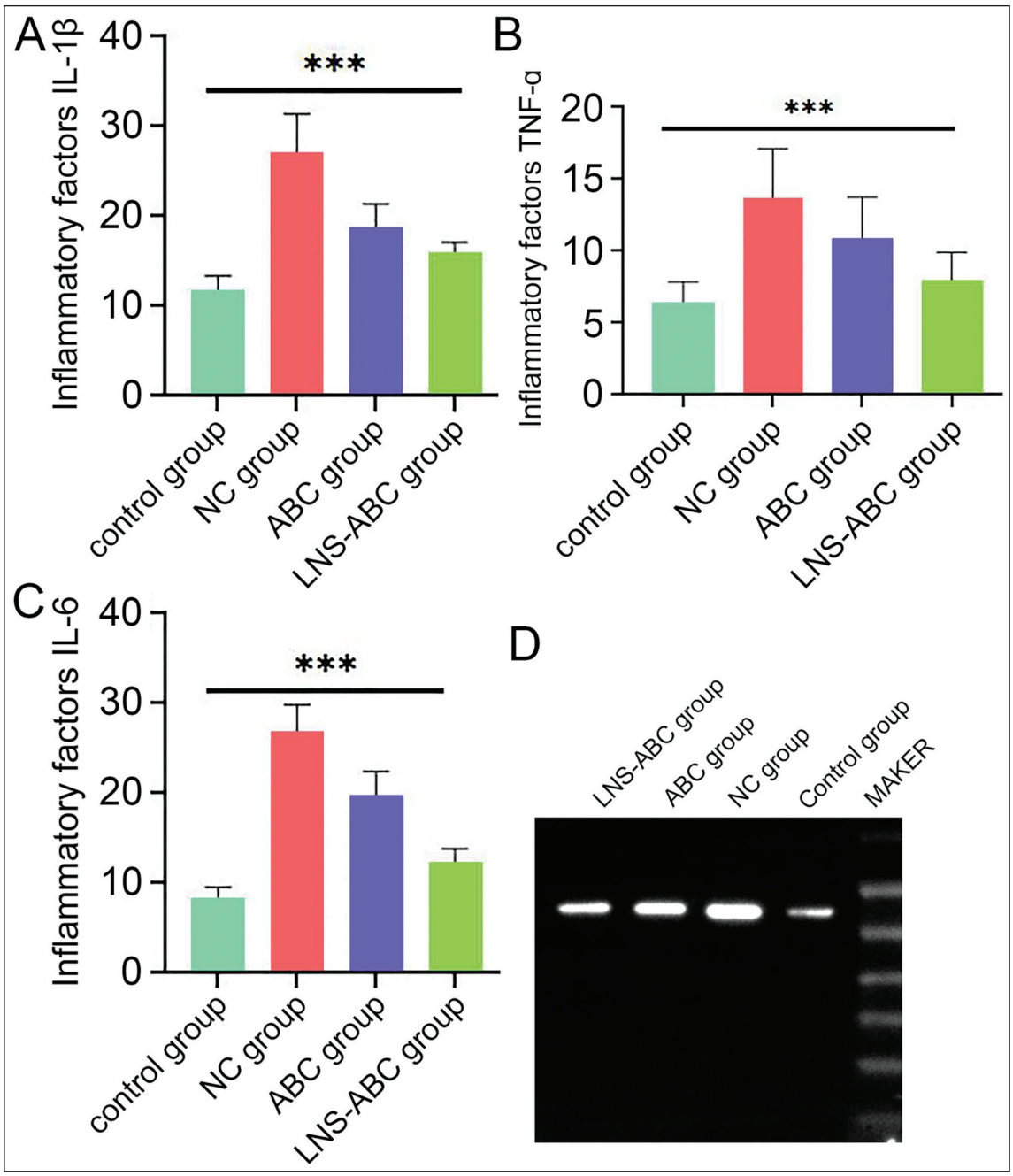


Figure 3. Expression of Inflammatory Factors in Acute Appendicitis.

Notes: (A) Interleukin (IL)-1β concentration (pg/mL); (B) tumor necrosis factor alpha (TNF-α) concentration (pg/mL); (C) IL-6 concentration (pg/mL); (D) signal transducer and activator of transcription 3 (STAT3) messenger ribonucleic acid (mRNA) quantitative reverse transcription polymerase chain reaction (qRT-PCR) detection. The error line represents the standard deviation (SD) for each set of data. All experiments were repeated three times. ***$p < .05$.

TNF-α, and COX-2, which inhibits cell infiltration and inflammatory factors production (Ma et al., 2021).

To assess the mechanism by which LNS-ABC improves the inflammatory response in mice with acute appendicitis, we used inhibitors and activators of STAT3 based on the intervention of LNS-ABC. We found that under the intervention of LNS-ABC+STAT3 agonist, inflammatory factors were significantly increased. On the contrary, inflammatory factors in the LNS-ABC+S3I-201 group were the lowest. It can be seen that STAT3 regulates the inflammatory response in LNS-ABC treatment. Specifically, when STAT3 agonists are used, the inflammatory response is exacerbated and the levels of inflammatory factors are significantly increased; while when STAT3 inhibitor (S3I-201) is used, the

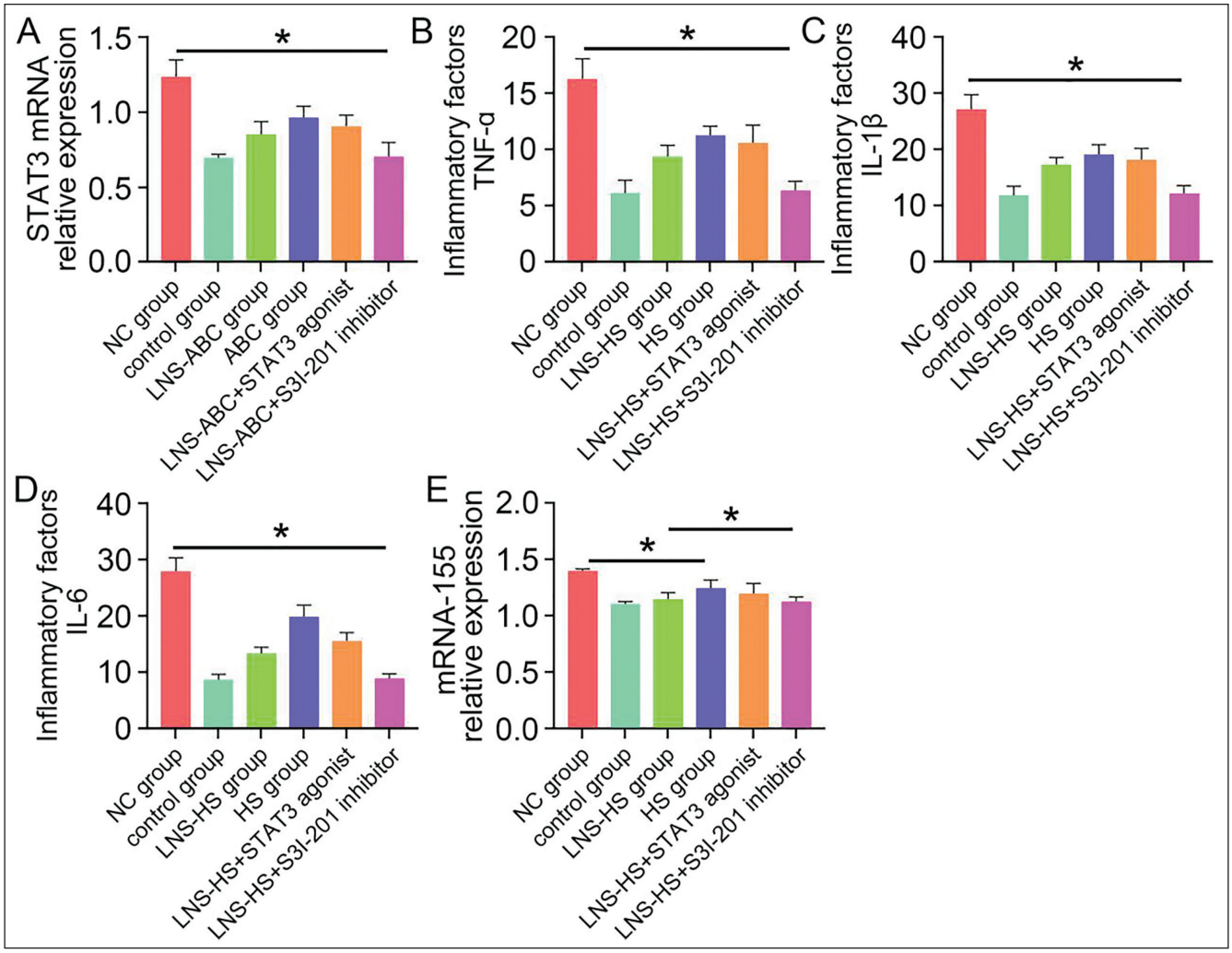


Figure 4. Baicalein Liposome Nanoparticles (LNS-ABC) Improves the Inflammatory Response in Mice with Acute Appendicitis, Regulates the Signal Transducer and Activator of Transcription 3 (STAT3) Signaling Pathway, and Has a Certain Relationship with miR-155.

Notes: (A) Relative expression of STAT3 messenger ribonucleic acid (mRNA); (B) tumor necrosis factor alpha (TNF-α) concentration (pg/mL); (C) interleukin (IL)-1β concentration (pg/mL); (D) IL-6 concentration (pg/mL); (E) the relative expression level of miR-155 ($2^{-\Delta\Delta Ct}$). The error line represents the standard deviation (SD) for each set of data. All experiments were repeated three times. *$p < .05$.

inflammatory response is significantly alleviated and the levels of inflammatory factors are reduced to the lowest state, indicating that inhibiting the activity of STAT3 may help reduce the inflammatory response, while activating STAT3 may aggravate the inflammatory response. Therefore, STAT3 may be an important therapeutic target, and the regulation of inflammatory response can be achieved by regulating its activity. Compared with LNS-ABC, it is further suggested that LNS-ABC can inhibit the STAT3 signaling pathway to improve inflammation in appendicitis. The results of this study showed that LNS-ABC blocked the nuclear translocation and DNA-binding ability of STAT3 by inhibiting the phosphorylation of STAT3, thus inhibiting the expression of downstream inflammatory genes. Compared with free baicalein, LNS-ABC showed a stronger anti-inflammatory effect, which may be due to its improved bioavailability and drug targeting. Liposome nanoparticles not only improve the water solubility of baicalein but also improve the therapeutic effectiveness by enhancing the osmotic and retention effect (EPR effect) of drug delivery to the site of inflammation.

Studies have shown that miR-155 can inhibit SOCS1 at a certain level, thereby enhancing the differentiation of Th1 cells and causing intestinal inflammation (Ma et al., 2021). In addition, it can also regulate the function of dendritic cells (DC), affect the balance of intestinal immune responses, and promote epithelial cell apoptosis by inhibiting the expression of Bcl-2, thus aggravating intestinal inflammation (Ma et al., 2021). We added miR-155 agonist and inhibitor, respectively, on the basis of LNS-ABC. The expression levels of related

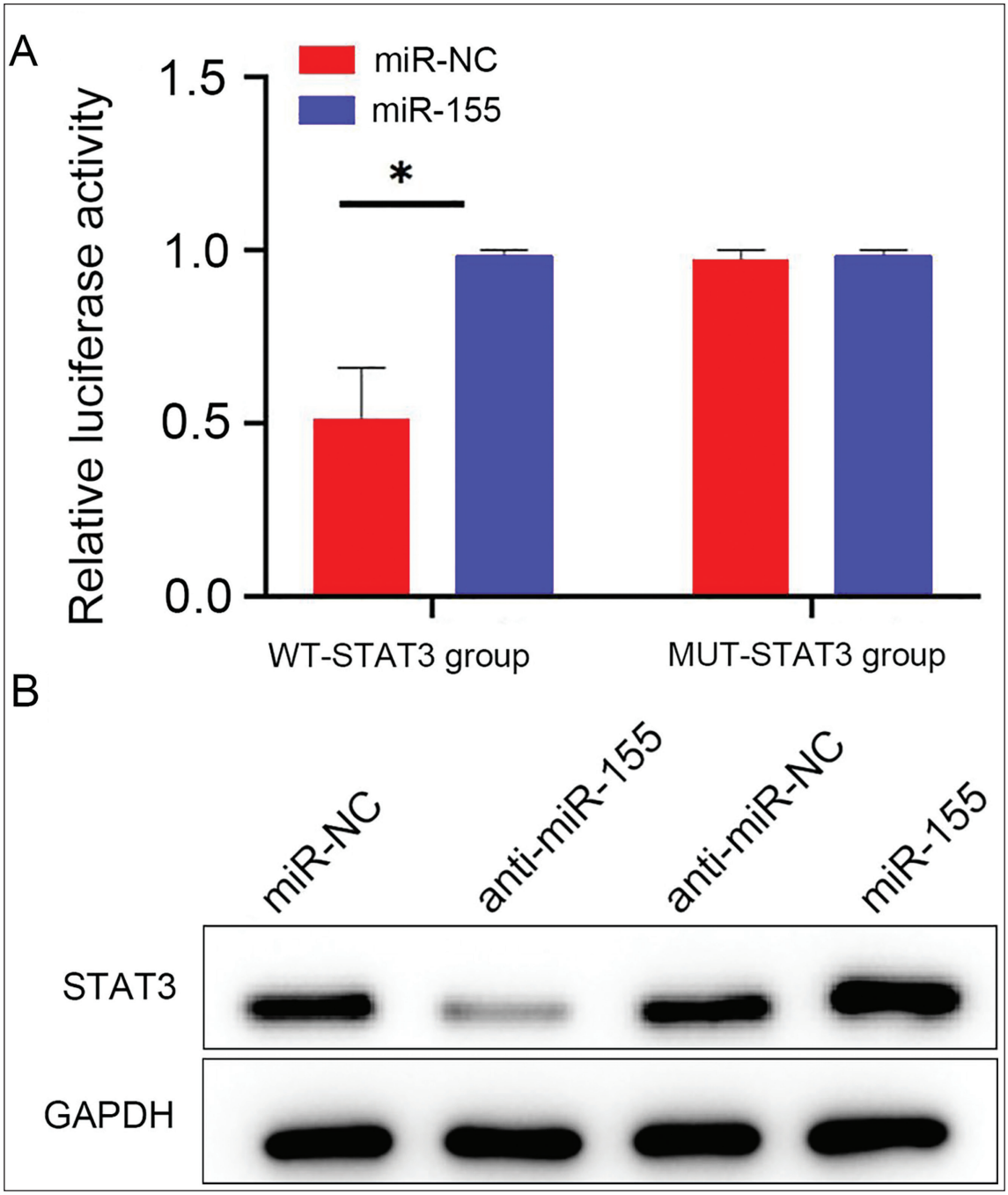


Figure 5. Signal Transducer and Activator of Transcription 3 (STAT3) and miR-155 Targeting.

Note: (A) Luciferase experiment; (B) the regulatory relationship between STAT3 and miR-155.

inflammatory factors in the LNS-ABC+miR-155 inhibitor group were similar to the trend of the above-mentioned LNS-ABC+STAT3 inhibitor group, while the LNS-ABC+miR-155 agonist group showed opposite results. This indicates that LNS-ABC inhibits inflammation by regulating the STAT3 signaling pathway and thereby inhibiting miR-155. This is because miR-155 can downregulate inflammation-related transcription factors such as AP-1 and NFAT5, thereby inhibiting MAPK signaling, blocking transmission of inflammatory signals, reducing the production of inflammatory factors, and improving inflammation (Yu et al., 2020). Further luciferase experiments confirmed that miR-155 directly targeted the 3′UTR region of STAT3 and inhibited its expression. On the contrary, activating miR-155 will aggravate the inflammatory response, which will help to further understand the regulatory effect and molecular mechanism of LNS-ABC

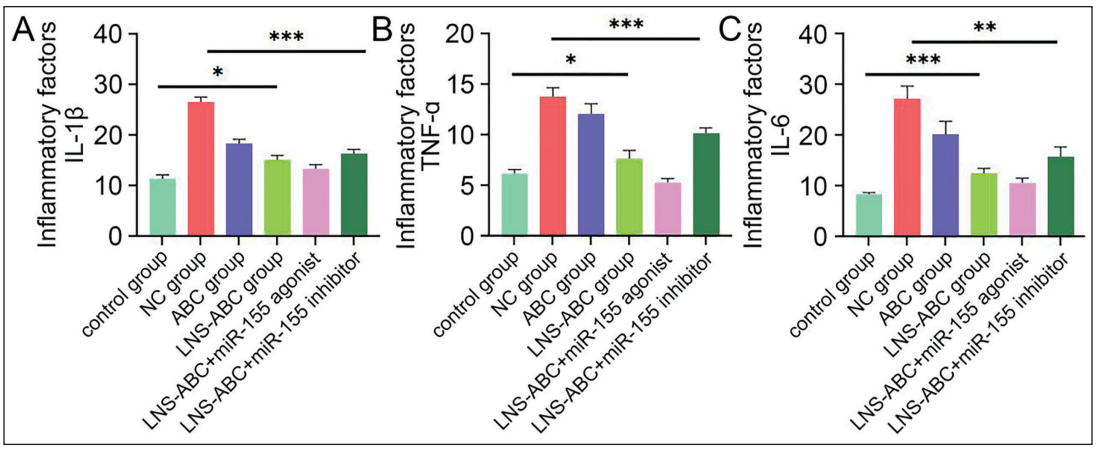


Figure 6. Under the Intervention of Baicalein Liposome Nanoparticles (LNS-ABC), miR-155 is Inhibited and Further Mediates the Suppression of Inflammatory Response.

Notes: (A) Interleukin (IL)-1β concentration (pg/mL); (B) tumor necrosis factor alpha (TNF-α) concentration (pg/mL); (C) IL-6 concentration (pg/mL). The error line represents the standard deviation (SD) for each set of data. All experiments were repeated three times. *$p < .05$, **$p < .01$, ***$p < .001$.

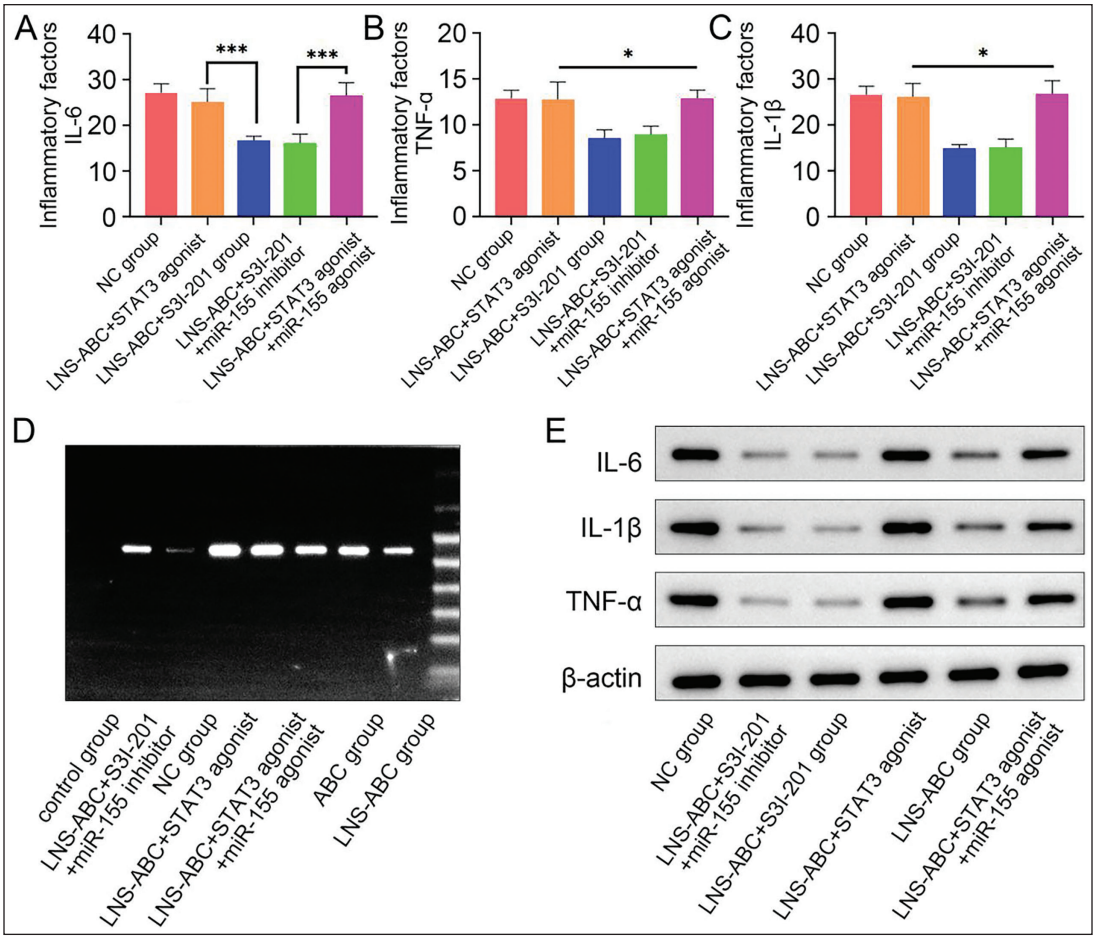


Figure 7. Baicalein Liposome Nanoparticles (LNS-ABC) Inhibits miR-155 Through the Intervention of the Signal Transducer and Activator of Transcription 3 (STAT3) Signaling Pathway, Thereby Improving the Inflammatory Response in Mice with Acute Appendicitis.

Notes: (A) Interleukin (IL)-6 concentration (pg/mL); (B) tumor necrosis factor alpha (TNF-α) concentration (pg/mL); (C) IL-1β concentration (pg/mL); (D) miR-135 level; (E) expression of various inflammatory factors. The error line represents the standard deviation (SD) for each set of data. All experiments were repeated three times. *$p < .05$, **$p < .01$, ***$p < .001$.

treatment on the inflammatory response. To verify this experimental result, STAT3 inhibitors were combined with miR-155 inhibitors on the basis of LNS-ABC intervention, and it was found that the levels of inflammatory factors in mice were significantly reduced, while STAT3 inhibitors and miR-155 inhibitors significantly reversed this trend. It was demonstrated that LNS-ABC inhibits miR-155 through the STAT3 signaling pathway. Our research also needs to further verify the *in vitro* stability and drug release kinetics of LNS-ABC and other parameters in the future. In acute appendicitis, upregulation of miR-155 leads to decreased STAT3 expression, thereby weakening the STAT3-mediated anti-inflammatory signaling pathway. By inhibiting the expression of miR-155, LNS-ABC restored the activity of STAT3, thereby inhibiting the inflammatory response. This discovery provides a new molecular target for the treatment of acute appendicitis.

Conclusion

In summary, LNS-ABC has good targeted medicinal value in acute appendicitis. It inhibits the STAT3 pathway and thereby inhibits the expression of miR-155 to a certain extent. It works by regulating and inhibiting the STAT3 signaling pathway, thereby inhibiting the expression of miR-155, improving inflammation in acute appendicitis, and has great potential in treating inflammatory responses in intestinal diseases. In this study, for the first time, LNS-ABC was associated with the STAT3/miR-155 pathway, filling the knowledge gap in this field. In addition, this study also revealed the regulatory role of miR-155 in acute appendicitis, providing a new direction for future research. However, there are still some shortcomings in this study: (a) This study only verified the effect of LNS-ABC in a mouse model, and its safety and efficacy need to be further verified in larger scale animal experiments and clinical trials in the future; (b) as for the specific regulatory mechanism of the STAT3/miR-155 pathway, more in-depth molecular biological studies are still needed to verify its function.

Abbreviations

ABC: Baicalein; LNS-ABC: Baicalein liposome nanoparticles; STAT3: Signal transducer and activator of transcription 3.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval and Informed Consent

This study has been pre-approved by the ethical committee of The Second Affiliated Hospital of Soochow University (No. 2022-0810). All subjects signed the consent forms before recruitment in this study.

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