Protective Effect of Dachengqi Decoction on Pancreatic Microcirculatory in Severe Acute Pancreatitis via Down-regulating HMGB-TLR-4-IL-23-IL-17A Mediated Neutrophil Activation Though Targeting SIRT1

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Research

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Abstract

Background

Dachengqi decoction (DCQD), one of classic prescription of Chinese herbal medicine has been widely used in clinic to treat severe acute pancreatitis (SAP). The damage of pancreatic microcirculation plays key pathogenesis of SAP. However, little is known about the molecular pharmacological activity of DCQD on pancreatic microcirculation in SAP. Therefore, the purpose of the study attempted to confirm the improvement of DCQD on pancreatic microcirculation is associated with suppressing neutrophil mediated immune-inflammatory response through promoting the inactivation of HMGB1-TLR-4-IL-23-IL-17A axis via targeting the SIRT1 signal pathway in SAP.

Material and Methods

Sodium taurodeoxycholate and cerulein were used to establish model of SAP in vitro and vivo, respectively. The pancreatic pathological morphology, wet weight ratio, myeloperoxidase (MPO) activity, cell viability and microcirculatory function of the pancreas, as well as serum lipase and amylase expressions were evaluated. The expression levels of SIRT1, acety-HMGB1, TLR-4, HMGB1, IL-23, IL-17A, neutrophil chemokines (KC, LIX, and MIP-2), and inflammation-related factors (IL-6, IL-1β, and TNF-α), the translocation of HMGB1 and the interaction of SIRT-HMGB1 in the pancreas and serum were determined by ELISA real-time PCR, western blotting and immunoprecipitation.

Results

In-vivo studies showed DCQD or neutralizing antibody (anti-23p19 or anti-IL-17A) could significantly decrease the activity of lipase, amylase, down-regulate the expression of CD68, MPO, wet/weight, IL-1β, IL-6, TNF-α,neutrophil chemokines (KC, LIX, MIP-2), alleviate pathological injury, and improve the microcirculatory function of the pancreas in rats with SAP. Moreover, DCQD remarkably augmented SIRT1 expression, promoted SIRT1 and HMGB1 combination, reduced HMGB1 translocation from nuclear to cytoplasm, and alleviated the expression of acetyl-HMGB1, HMGB1, IL-17A, TLR-4 and IL-23 in vitro and vivo with SAP. However, the intervention with EX527 (SIRT1 inhibitor) or r-HMGB1 (recombinant HMGB1) could obliviously reverse the above-mentioned influence of DCQD in SAP. In vitro, we confirmed that DCQD could decrease the acetylation, migration and release of HMGB1, and improve the decline of cell viability, SIRT1, SIRI-HMGB1 combination induced by cerulein with promoting macrophage to release IL-23 through HMGB1/TLR-4.

Conclusion

DCQD treatment improves SAP-induced pancreatic microcirculatory dysfunction by inhibiting neutrophil-mediated inflammation through the inactivation of HMGB1-TLR-4-IL-23-IL-17A signaling via Targeting SIRT1.

Trial registration: No. 365, 2020.

1. Introduction

Severe acute pancreatitis, one of acute abdomens in clinic, accounts for 10 – 20% of all acute pancreatitis cases [1]. It is characterized by rapid onset, fast progression, and poor prognosis [1]. Moreover, it causes inflammatory cascade and organ failure in its early stages resulting in high mortality [1].Therefore, SAP is a great threat to human health, life, and the economy [1]. Despite extensive research and efforts to develop pharmacological tools, currently no specific treatments for SAP exist [1]. Therefore, determining the pathophysiological mechanisms underlying SAP is crucial to identify effective therapeutic drugs and preventive measures for SAP.

The pathogenesis of SAP is complex and involves in the abnormal activation of pancreatic inflammatory mediators, microcirculation dysfunction, translocation of intestinal flora, apoptosis, and oxidative stress; additionally, the immune inflammatory response mediated pancreatic microcirculation dysfunction is always active, which is also an important molecular biomarker to determine the outcome of pancreatitis [2]. Notably, the activation of immune inflammatory response is relied on the recruitment and migration of neutrophil in SAP [3]. Therefore, it is an attractive treatment strategy to improve SAP by suppressing the inflammatory response mediated pancreatic microcirculation dysfunction through promoting the inactivation of neutrophil.

HMGB1, one of endogenous molecule secreted from apoptotic and necrotic cell possess a pivotal influence in the development of SAP by activating immune response [2]. Once the pancreatic cells are harmfully stimulated , the HMGB1 in the nucleus will be modified by acetylation and then released to the extracellular space where it pairs with receptors(TLR-4) on the surface of macrophages to induce the TLR-4-mediated immune responses[2,4]. This further promotes the releasing HMGB1 and amplifies the immune reaction. It is worth noting that HMGB1-TLR-4 signaling stimulates macrophages to secrete the pro-inflammatory cytokine IL-23 and IL-23 can promote Th17, γ δ T, NK and NKT cells to secrete IL-17A to regulate the activation of neutrophil [5]. In addition, the activity and nucleocytoplasmic shuttle of HMGB1 are dependent on the acetylation degree of HMGB1. SIRT1, one of the key acetylation modifying enzymes possess the ability to suppress HMGB1 transcription by deacetylation of HMGB1. Therefore, it is an effective strategy to alleviate TLR-4-mediatted the activation of neutrophil by triggering the SIRT1/HMGB1 signaling in the development of SAP [6].

Dachengqi decoction (DCQD) is a traditional decoctions widely used in the clinic for SAP treatment [7-8]. Its active ingredients include rhubarb, mirabilite, Fructus aurantii, and *Magnolia officinalis* [7]. DCQD seems to be positively associated with improving microcirculation of pancreas in SAP [8-9]. However, it is a pity the concrete mechanism that sustains the influence DCQD on microcirculation is not clear up to date. Therefore, the purpose of the study attempted to confirm the improvement of DCQD on pancreatic microcirculation is associated with suppressing neutrophil mediated immune inflammatory response though promoting the inactivation of HMGB1-TLR-4-IL-23-IL-17A axis via targeting SIRT1 signal pathway in SAP.

2. Material And Methods

2.1. Drugs, reagents, instruments and animals,

DCQD is consisted of Houpu, Da Huang, Mangxiao and Zhi Shi .The above herbs were acquired from Lv Yuan Pharmaceutical Co, Ltd in Chengdu. The finally collected DCQ granules were dissolved in saline to obtain the DCQD (2 g/ml). The concrete specific compatibility, steps, extraction and use was according to the previous article [9].

Reagents were obtained from the following sources: sodium taurocholate, cerulein (Shanghai Dibao Biology Technology Co., Ltd); lipase, and amylase, HMGB1, TNF-α,IL-23, IL-1β, KC, IL-17A,LIX, IL-6 and MIP-2 ELISA Kits (Biyuntian); TLR-4, HMGB1 and Acety-HMGB1 antibodies (Abgent, USA); Myeloperoxidase (MPO) and CD68 Colorimetric Activity Assay Kit (Biovision, USA).EX527, r-HMGB1, neutralizing IL-23p19 and IL-17A (Biolegend, San Diego, CA) were purchased from Sigma

The following instruments were purchased: enzyme labeling instrument, two-dimensional electrophoresis instrument, PCR (Bio-Rad, USA), paraffin embedding and pathological section machines (Leica, Germany), real-time PCR (Applied Biosystems, USA), Western blot imager (Pei Qing, Shang Hai).

70 male healthy SD rats (weighing 220 ± 30 g) were obtained from Sichuan University. The rats were located in a standard SPF environment with humidity of 60 ± 4 % and a temperature of 22 ± 2 °C. The experiments were approved by the Sichuan Province Hospital2011220, and the experimental protocols were strictly carried out one the base of NIH Guidelines.

2.2 Animal grouping, model establishment, and intervention

The 70 rats were randomly separated into seven groups: Sham, SAP, Dachengqi decoction (DCQD), EX527, anti-IL-17, recombinant HMGB1(r-HMGB1) and anti-IL-23p19 groups (n =10). The rats were fed *ad libitum* for 7 days before establishing the SAP model.

The rats in the SAP, DCQD, EX527, anti-IL-17, r-HMGB1, and anti-IL-23p19 groups were anesthetized with pentobarbital sodium (40 mg/kg). An upper abdominal incision was made by cutting open the skin and muscle to expose the bile duct, which was temporarily clamped at a small artery on the portal side of the liver. The insulin injection needle was stretched into biliopancreatic duct by means of the nipple, and sodium taurocholate (1mg/kg) got into the biliopancreatic duc by injection. After 2h of injecting sodium taurocholate, the animal in the EX527, DCQD and r-HMGB1 group were treated with DCQD using an oral gavage at a dose of 20 g/kg at 2h after operation [9]. The animals in the EX527 and r-HMGB1 group were also treated with EX527 (10mg/kg) or r-HMGB1 (100μg /kg) by intraperitoneal injection in according with previous article [10, 11]. The rats in the anti-IL-17A groups and anti-IL-23p19 group were treated with neutralizing antibody against IL-17A (1mg/kg) and IL-23p19 (0.2 mg/kg) ,respectively by intraperitoneal injection in according with previous article [12, 13]. After 12 h, the rats in all groups were euthanized with pentobarbital sodium and the serum and pancreas tissue were collected for further study.

2.3 Cell culture

The rat pancreatic acinar cells lines (AR42J) and RAW264.7 were acquired from ATCC (Rockville, MD, USA). After 30 min treatment without or with DCQD, AR42J cells were co-incubated with cerulean for 24 h.

2.4 Gene silencing

AR42J cells were transfected with control siRNA or TLR-4. The RAW 264.7 cells were intervened with rHMGB1.

2.5 TLR4 overexpression

RAW 264.7 cells were designated to transfect TLR4 and control genes by use of Lipofectamine2000 in line with the instruction. The TLR4 and IL-23 expression were examined after transfection at 24 h.

2.6 Assaying Cell viability

MTT was used to assay cell survival and the concrete steps were on the base of instruction. The AR42J cells were preconditioned with or without DCQD EX527 or r-HMGB1 (0.004 g/mL) , successively 24 co-incubation with cerulein 10nmol/l .

2.7 Microcirculation of the pancreas

Each rat was injected with 1.5 ml FITC-RBC via tail vein. Measuring flow velocity, functional blood vessel number, red blood cell flow and blood vessel number to evaluate microcirculation of the pancreas by using analysis system of BI-2000 medical image.

2.8 Histology and wet /weight

Excised pancreatic tissue was sectioned and mounted in paraffin, following performing hematoxylin and eosin (HE) staining to evaluate pancreatic pathological morphology. The pancreas was assayed under a light microscope. The pancreatic tissue was graded by evaluating necrosis+ hemorrhage+ edema +inflammation according to the modified Kusske scoring standard [9]. No inflammatory cell infiltration, hemorrhage, edema and necrosis were scored 0; necrosis area (1% - 10%), hemorrhage and edema(0-25%) were 1 point; necrosis area (11% - 20%), hemorrhage and edema (25% - 50%)were 2 points; necrosis area (21% - 30%), hemorrhage and edema (50% - 75%) were 3 points; necrosis area(> 30%) hemorrhage and edema (> 75%)were 4 points. 0.5 points for 5 inflammatory cells and 4 points for more than 30 inflammatory cells. The weight index of pancreas was calculated as the pancreas weight (g) / body weight (g) x100%.

2.9 Amylase and lipase activity assays in serum

The collecting blood was centrifuged to get the supernatant. Th amylase and lipase activity were assayed by exploiting activity colorimetric kits for amylase and lipase following the manufacturer's instructions.

2.10 Assaying IL-23, HMGB1, IL-1β, IL-17A, MIP-2, TNF-α, LIX, IL-6, and KC

The concentrations of IL-23, HMGB1, IL-1β, IL-17A, MIP-2, TNF-α, LIX, IL-6, and KC in the pancreas and serum were determined using rat ELISA kits for each protein following the manufacturer's instructions.

2.11 Detection of IL-23, IL-1β, IL-17A, MIP-2, TNF-α, LIX, IL-6, and KC mRNA expression in the pancreas

Total RNA were extracted by use of TRIzol method after homogenization of the frozen pancreas in liquid nitrogen. Real-time PCR was exploited to detect the mRNA expression. The concrete steps were in line with instructions. The primer sequences are displayed in Table 1.

2.12 Examining SIRT1 activity

The SIRT1 activity was evaluated by Fluorescent Assay Kit for SIRT1 Activity on the base of instructions.

2. 13 Western blotting

Total, nuclear and cytoplasmic proteins were extracted from pancreatic tissues by lysis, centrifugation, and quantification. Western blot analysis was performed using SIRT1 (1:400), Acey-HMGB1 (1:400), TLR-4 (1:1000), HMGB1 (1:400), β-actin (1:4000) and Histone (1:1000). Signals were detected by incubation with secondary antibody, followed by exposure to the ECL-Plus reagent.

2.14 MPO and CD68 assay

Immunohistochemistry was used to evaluate the MPO and CD68 expression in pancreatic tissues.

2. 15 Statistical methods

All documents are displayed as mean ± SEM. T-test and One-way ANOVA were used to assay multiple comparisons. Tukey's post-hoc test was exploited to evaluate individual means. $P<0.05$ were considered to have significance.

Table1: The primer for IL-23, IL-17A, KC, LIX, MIP-2, IL-6, IL-1β, and TNF-α

Gene	Sense group(5'-3')	Antisense group(5'-3')
HMGB1	ATGGGCAAAGGAGATC	ATTCATCATCATCATCTTCT
IL-17A	GGAAAGCTGGAC-CACCACA	CACACCCACCAGCATCTTCTC
IL-23	AGGACTTGTGCTGTTCTTGTTTTGT	CTCTGGGGTTTGTTTCTTTTCTCTT
KC	TCACGCTTCTGGGCCTGTTG	CAGCCGACTCATTGGGATCATC
LIX	TCACGCTTCTGGGCCTGTTG	CAGCCGACTCATTGGGATCATC
MIP-2	GGCAAGGCTAACAGACCTGGAAAG	CACATCATCAGGTACGATCCAGGCTTC
IL-6	TGCGCTGGGCTTAGATCATT	TGGATGCCTTTTATGTCGTCT
IL-1β	AGGGAAATCGTGCGTGACAT	GAACCGCTCATTGCCGATAG
TNF-α	ACCAAGGATGAGGGCGACTA	CAGGCTTATGCCACCACACTT
β-actin	CATCCGTAAAGACCTCTATGCCAAC	ATGGAGCCACCGATCCACA

3. Results

3.1 Wet /weight of the pancreas, activity of serum amylase and lipase, cell viability and survive rates.

In comparison with the SAP group, the wet /weight, activity of amylase and lipase, and mortality in the Sham, DCQD, anti-IL-17A and anti-IL-23-p19 groups were obliviously decreased ($P < 0.05$). In comparison with the DCQD group, the wet /weight, activity of amylase and lipase, and the mortality in the markedly increased ($P < 0.05$) (Figure1A-C)

To simulate SAP in rats, AR42J cells were treated with Cerulein. As displayed in Figure 2C–D, In comparison with the SAP group, the cell viability in the Sham, anti-IL-17A, DCQD and anti-IL-23-p19 groups were significantly up-regulated ($P < 0.05$). In comparison with the DCQD group, the cell viability in the EX527 and r-HMGB1 groups were remarkably up-regulated ($P < 0.05$) (Figure1D-E) ($P < 0.05$).

3.2 Pancreatic histopathological morphology and the expression of CD 68 and MPO in the pancreas

HE and immunohistochemistry were used to assess pancreatic histological morphology, and CD 68 and MPO expression, respectively. The Sham group displayed the pancreas of some of the rats had a slight edema, a small amount of inflammatory infiltration, but no necrosis or hemorrhage. The pancreatic tissues from SAP group had seriously damaged pancreatic tissue, widespread edema and hemorrhage, a large number of inflammatory cells infiltration and acinar cells necrosis with higher pathological injury scores than Sham group ($P < 0.05$). The pancreatic tissues from rats in the anti-IL-23-p19, DCQD and anti-IL-17A groups had slightly edema, a small amount of inflammatory infiltration, hemorrhage and necrosis with lower pathological injury scores than SAP group ($P < 0.05$). In comparison with the DCQD group, the r-HMGB1 and EX527 groups had more pancreatic tissue necrosis, inflammatory cell infiltration, edema and hemorrhage with higher pathological injury scores ($P < 0.05$) (Figure2).

In comparison with the sham group, the SAP group has higher CD68 and MPO expression ($P < 0.05$). In comparison with the SAP group, the DCQD group, anti-IL-17A and anti-IL-23-p19 groups have lower CD68 and MPO expression ($P < 0.05$). In comparison with the DCQD group, the EX527 and r-HMGB1 have higher CD68 and MPO expression ($P < 0.05$) (Figure2).

3.3 Microcirculatory function of the pancreas

Impaired microcirculation of the pancreas is a typical clinical manifestation of severe pancreatitis [2-3]. In comparison with the sham group, the SAP group has lower blood flow velocity, number of functional blood vessel, RBC flow and number of blood vessel ($P < 0.05$). Compared to SAP group, the DCQD, anti-IL-17A and anti-IL-23-p19 groups had blood flow velocity, number of functional blood vessel, RBC flow and number of blood vessel ($P < 0.05$). Moreover, The r-HMGB1 and EX527 groups had higher blood flow velocity, number of functional blood vessel, RBC flow and number of blood vessel than DCQD group ($P < 0.05$) (Figure3).

3.4. Expression of IL-23, HMGB1, IL-1β, IL-17A, TNF-α and IL-6

The immune inflammatory response possesses a key role in SAP progression [2-3]. Therefore, the study examined the level of IL-23, HMGB1, IL-1β, IL-17A, TNF-α and IL-6 in the serum and pancreas at both gene and protein levels. The SAP group has higher level of HMGB1, IL-17A, IL-23, IL-6, TNF-α and IL-1β in the serum and pancreases than Sham, DCQD, anti-IL-17A and anti-IL-23-p19 groups ($P < 0.05$). Moreover, EX527 and r-HMGB1 group have higher level of IL-23, HMGB1, IL-1β, IL-17A, TNF-α and IL-6 in serum and pancreas at gene and protein levels compared to DCQD group ($P < 0.05$) (Figure4).

3. 5 Neutrophil activation

MPO is a surface marker of neutrophils, and MIP-2, LIX and KC are neutrophil chemokines that reflect neutrophil activation [14]. Therefore, we measured the pancreatic MPO activity, and the protein and mRNA levels of MIP-2, LIX and KC in the serum and pancreas.. The SAP group has higher pancreatic MPO activity and more expression of MIP-2, LIX and KC compared to the sham group, DCQD, anti-IL-17A and anti-IL-23-p19 groups ($P < 0.05$), Moreover, The r-HMGB1 and EX527 groups have higher pancreatic MPO activity and more expression of MIP-2, LIX and KC compared to the DCQD group ($P < 0.05$)(Figure 5).

3.6. The SIRT1, acety-HMGB1 and TLR-4 expression, HMGB1 translocation and SIRT1-HMGB1combination in pancreas

HMGB1-TLR4 signal pathway have significant influence in SAP and the activation of HMGB1can be regulated by SIRT1 though deacetylation [7-8]. Therefore, we examined the activity of SIRT1, combination of SIRT1 and HMGB1, and the level of SIRT1, acety-HMGB1**,** TLR4 and the mitigation of HMGB1from nuclear to cytoplasm in the vitro and vivo. The SAP group had higher acety-HMGB1**,** TLR-4 and cytoplasmic HMGB1 expression than sham group with lower SIRT1 activity, less level of nuclear HMGB1, SIRT1 and lower SIRT1- HMGB1 combination ($P < 0.05$), while the DCQD group showed an increase on the activity of SIRT1, combination of SIRT1 and HMGB1, and level of SIRT1 and nuclear HMGB1 with a

decrease on the level of acety-HMGB1, TLR-4 and cytoplasmic HMGB1 than SAP group ($P < 0.05$). Moreover, the EX527 group displayed higher level of acety-HMGB1, TLR-4 and HMGB1 in cytoplasm than DCQD group with less SIRT1- HMGB1 combination, lower expression of SIRT1 and nuclear HMGB1 and lower SIRT1 activity ($P < 0.05$) (Figure6).

3.7 The release of IL-23 though relying on HMGB1/TLR-4 way in SAP

As shown in Figure7 A–B, r-HMGB1 up-regulated IL-23 secretion and increased the IL-23 and TLR-4 levels ($P < 0.5$). In comparison with the SAP group, the DCQD and TLR-4 siRNA groups has lower expression on IL-23 and TLR-4. However, overexpression TLR-4 can up-regulate IL-23 and TLR-4 expression by using a TLR-4 expression plasmid as demonstrated higher IL-23 and TLR-4 expression in plasmid TLR-4 group compared to SAP group ($P < 0.05$).

3.8 The signal pathway of DCQD on pancreatic microcirculatory in SAP (Figure8)

4. Discussion

SAP, one of common clinical critical illness, easily caused multiple organ failure and frequent death [1]. In this study, we used sodium taurodeoxycholate or cerculein to induce SAP in vivo and in vitro respectively. In vivo, SAP caused a disturbance in the microcirculation of the pancreas, an increase in serum amylase and lipase activity, and an increase in pancreas wet weight ratio. In vitro, cerculein can significantly decrease the cell viability of AR42J, which are in line with previous studies [9]. These indicated that the SAP models of in vivo and in vitro were successfully established. We also displayed that DCQD, anti-IL-17A or anti-IL-23-p19 treatment remarkably decreased lipase and amylase activity, reduced pancreatic pathological injury and dysfunction of microcirculatory, downregulated the weight index, and improved cerculein -induced the decline on the cell viability of AR42J. Moreover, both r-HMGB1 (a HMGB1 agonist) and EX527 (SIRT1 inhibitor), effectively abated the protective effect of DCQD in SAP. The findings were supported as showed higher serum activity of lipase and amylase, more serious pancreatic pathological injury, weight index and cell viability, and worse microcirculatory function in EX527 and r-HMGB1 groups than DCQD group. Furthermore, previous studies have confirmed that activating HMGB1 and IL-23/IL-17A pathway significantly aggravated pancreatic microcirculatory impairment. Therefore, our study further confirms that the protective effect DCQD on SAP was achieved though down-regulating HMGB1-mediated pancreatic microcirculatory dysfunction and IL-23/IL-17A signal pathway play an axis on regulating microcirculatory dysfunction in SAP.

Increasing evidence showed that the inflammatory response possesses significant influence in controlling the evolvement of pancreatic microcirculatory dysfunction in SAP by producing various inflammatory cytokines [6, 14]. TNF-α, IL-6 and IL-1β are the most important symbol of inflammatory cytokines in SAP [14]. TNF-α is an inflammation-initiating factor that can mediate the release of IL-6 and other inflammatory cytokines, stimulate the oxygen free radicals and nitric oxide production, promote leukocyte chemotaxis and adhesion, and damage pancreatic tissue[15]. IL-6 possesses critical influence

in setting off immune response in SAP, and is mainly released by macrophages, T lymphocytes, dendritic cells and T cells [14-15]. IL-1β is deeply participated in the destruction of pancreatic tissue and the formation of edema in SAP [15]. In this study, intervention with anti-IL-17A, DCQD or anti-IL-23p19 significantly decreased TNF-α, IL-6 and IL-1β expression. Nevertheless, r-HMGB1 and EX527 can abolish the influence of DCQD on inflammatory response in SAP as confirmed higher expression of TNF-α, IL-6 and IL-1β in r-HMGB1 and EX527 groups than DCQD group. Above results implied DCQD could cut down SAP-induced microcirculatory dysfunction through inhibition of HMGB1-mediated inflammatory response and IL-23/IL-17A signal pathway can improve microcirculatory dysfunction by attenuating inflammation in SAP.

Interestingly, blocking neutrophil activation has previously been demonstrated that can suppress inflammatory response in SAP [7]. Therefore, the intensity of the inflammatory response depends on neutrophil activation in SAP. In addition, MPO is a surface marker of neutrophils, and KC, LIX, and MIP-2 are neutrophil chemokines that reflect the activation of neutrophil by recruitment and migration of neutrophil. Anti-IL-23-p19, DCQD, or anti-IL-17A treatment decreased pancreatic MPO expression, activity and the level of MIP-2., KC and LIX. Nevertheless, r-HMGB1 intervention can cut down the negative influence of DCQD on the inactivation of neutrophil and lessening neutrophil chemokines as showed higher MPO activity and higher expression of MIP-2., KC and LIX in r-HMGB1 group than DCQD group. These findings further demonstrated that anti-IL-23-p19, DCQD, or anti-IL-17A cut down inflammatory response though inactivation of neutrophils in SAP

IL-17A is a common molecule that critically modulates host defense against detrimental inflammatory stimulation [6]. Controlling the activation and recruitment of neutrophils to the pancreas is the main mechanism of IL-17A activity [7]. IL-17A is mainly released from NKT cell, γδ T cell and Th17 cells, etc. [6, 15]. Notably, the release of IL-17A is controlled by the heterodimeric cytokine IL-23 through the following mechanism [16-17]: IL-23 trigger naïve CD4 + T cells differentiation into Th17, and subsequently stimulates NKT cells to release IL-17A, along with anti-CD3. Additionally, IL-23 promotes to release IL-17A from γδ T cells through working with IL-1 [17]. In our study; SAP can stimulate activating IL-23/ IL-17A signaling and DCQD significantly promoted inactivation of IL-23/ IL-17A axis as showed lower level of IL-17A and IL-23 in DCQD group than SAP group. However, r-HMGB1 can boost activating IL-23/IL-17A signaling in SAP as demonstrated higher IL-17A and IL-23 level in r-HMGB1 group than DCQD group. Moreover, we also demonstrated neutralizing IL-23 cut down IL-17A expression in SAP that further confirmed IL-23 could promote IL-17A secretion. These outcomes were in agreement with previous research that implied the inhibitory effect of DCQD in the inactivation of neutrophils is though inactivation of IL-23/ IL-17A axis though HMGB1

HMGB1 is a non-DNA binding protein in the eukaryotic cell nucleus and is widely distributed in the pancreas, lung, liver, kidney, lymph, and other tissues [2]. It is known to function as a DAMP, interacting with RAGE and TLR members [2]. In our study, SAP has only influence on the TLR-4. This is inconsistent past research that may be attributed to drug dosage, purity, technology, individual differences in rats.TLR-4 is a pattern-recognition receptor (PRR) and it has been extensively validated that TLR-4 mediates

HMGB1-induced pancreatic injury in SAP [2]. A previous research displayed that HMGB1 stimulates TLR-4 expression in the pancreases, which is positively related to the promotion of macrophages and dendritic cells to secrete IL-23 in SAP [8]. Blocking TLR-4 can trigger the inactivation of IL-23/IL-17A signaling [18]. In our vivo study, DCQD treatment remarkably advanced the inactivation of HMGB1/TLR-4 signal pathway as displayed less expression of TLR-4 and CD68, and lower acetylation, migration and release of HMGB1. Moreover, r-HMGB1 can abolish the impact of DCQD in signal pathway of HMGB1/TLR-4 in SAP as confirmed higher expression of TLR-4 and CD68, and more acetylation, migration and release of HMGB1 in r-HMGB1 group than DCQD group.

In vitro studies further confirmed DCQD could decrease the secretion of HMGB1 and HMGB1 can promote macrophage to secrete IL-23, and increase IL-23 and TLR-4 expression. In addition, SiRNA TLR-4 can advance the inactivation of macrophages and alleviate IL-23 and TLR-4 expression. However, overexpressing TLR-4 has the converse influence as confirmed higher LR-4 and IL-23 expression in plasmid-group than SAP group. These above results indicated that the negative effect of DCQD on activating IL-23/IL-17A signaling is though promoting the inactivation of HMGB1-TLR-4 signal pathway via deacetylation of HMGB1.

Noteworthy, the degree of deacetylation of HMGB1 is dependent on SIRT1, one of NAD + dependent histone deacetylase, which can deacetylate the activities of HMGB1 though interacting with HMGB1 at promoter region [19]. In the study, DCQD can suppress the sodium taurocholate or cerulean-induced the decrease on the SIRT1 activity, expression and the interaction of HMGB1. However, EX527 can abolish the influence of DCQD on SIRT-HMGB1 axis in SAP. This indicated that DCQD can alleviate acetylation of HMGB1 though regulating SIRT-HMGB1signal pathway in the SAP.

Conclusion

In summary, our study provides compelling evidence the protective effect of DCQD on pancreatic microcirculatory in SAP is though inhibiting neutrophils-mediated inflammatory reactions. The above action mechanism of DCQD is likely associated with inactivation of HMGB1-TLR-4-IL-23-IL-17A axis via targeting SIRT1. However, this study was only performed non-knockout mice with a small sample size. Further study is required with larger sample sizes and knockout mice as well as clinical observations and more cellular intervention to confirm the efficacy and mechanism of DCQD in SAP.

Abbreviations

DCQD, Dachengqi decoction; SAP, severe acute pancreatitis; MPO, myeloperoxidase; SIRT1, Sirtuin1; r-HMGB1,recombinant HMGB1; HMGB1,high mobility group protein 1; KC, cytokine-induced neutrophil-attracting chemokine; LIX,LPS-induced chemokine;MIP-2, Chemokine Ligand 2;

Declarations

Availability of materials and data

The materials and data used in the current study are all available from the corresponding author upon reasonable request

Ethics approval and consent to participate

The study is authorized, approved and rigorously followed by Sichuan province hospital ethics committee. Ethical review number is No. 365, 2020

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no competing interests.

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Author contributions

Jia Wang wrote the manuscript. Pen lei collected the data. Da-qing Hong provides suggestion. Jiong zhang devised the article.

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Figures

Figure1

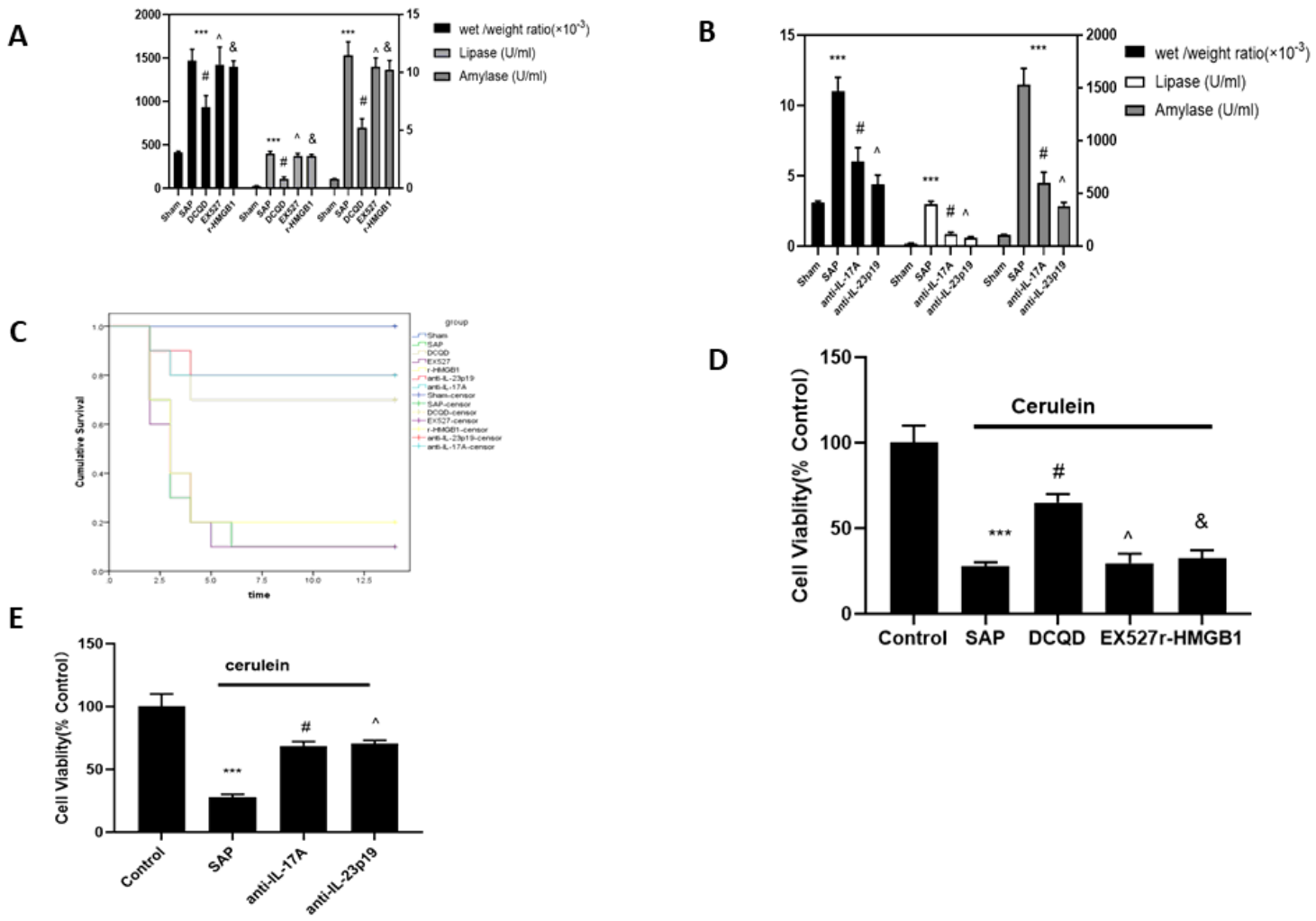

Figure 1

The effect of DCQD, EX527, anti-IL-17A, r-HMGB1, and anti-IL-23p19 on wet/weight, amylase and lipase activity, survive rats, and AR42J cell viability in SAP A: The influence of DCQD, EX527 and r-HMGB1 on wet/weight, amylase and lipase activity; B: The influence of anti-IL-17A and anti-IL-23p19 on wet/weight, amylase and lipase activity : C: The influence of DCQD, EX527, anti-IL-17A, r-HMGB1 and anti-IL-23p19 on the survive rats in SAP; D: The effect of DCQD, EX527 and anti-IL-17A, r-HMGB1and anti-IL-23p19 on the AR42J cell viability in SAP; E:The effect of anti-IL-17A and anti-IL-23p19 on the AR42J cell viability in SAP; *** P < 0.001 (SAP vs. Sham); #P < 0.05 (SAP vs. DCQD); ^P < 0.05 (EX527vs. DCQD); &P < 0.05 (r-HMGB1vs. DCQD);^P <0.05 (anti-IL-23p19 vs SAP); #P <0.05 (anti-IL-17A vs SAP); *** P < 0.001 (SAP vs. Control);

Figure2

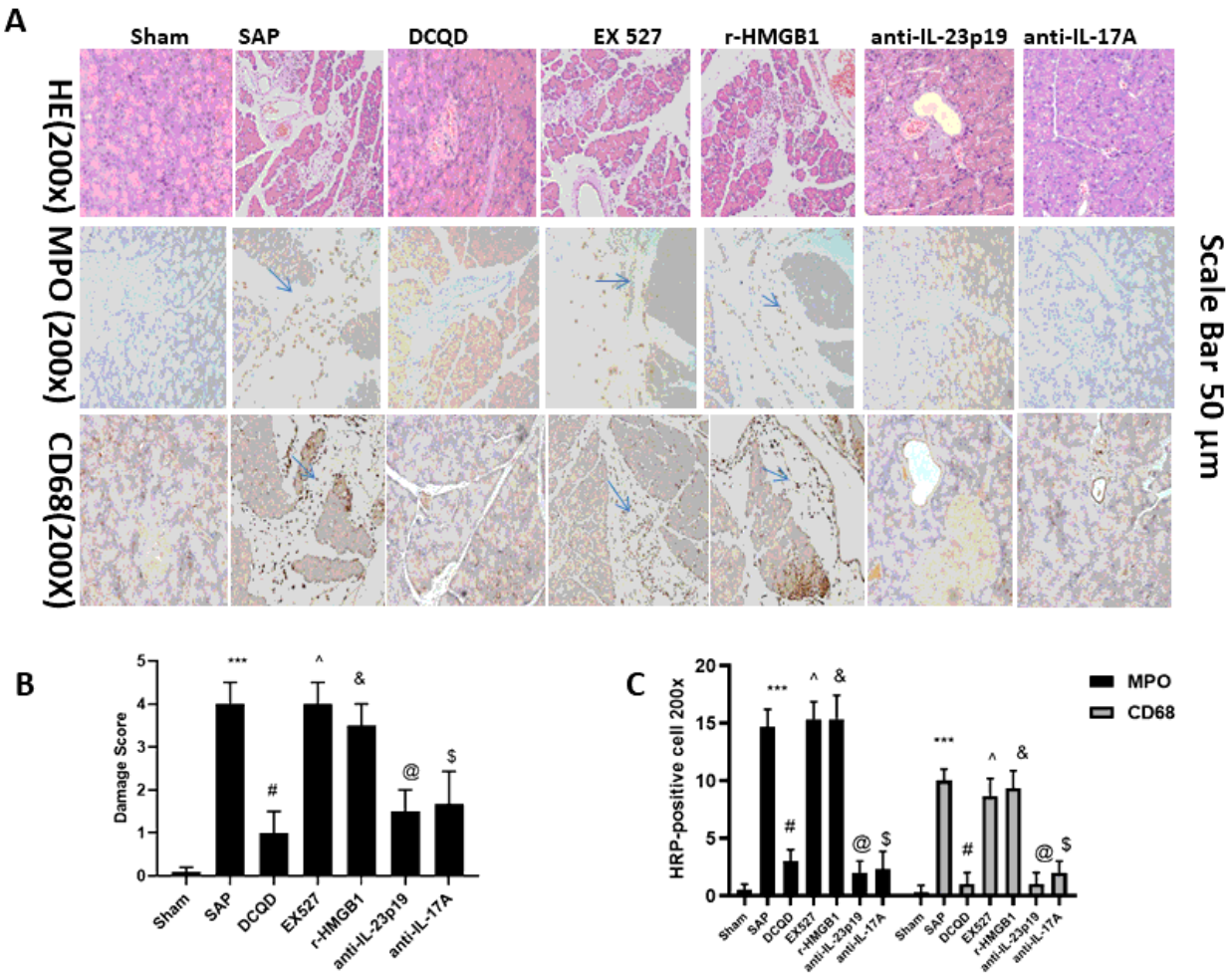

Figure 2

The influence of DCQD, EX527, anti-IL-17A, r-HMGB1 and anti-IL-23p19 on pancreatic histopathological morphology, and CD68 and MPO expression in SAP A: Representative results for HE staining histopathological morphology of pancreas and immunohistochemistry for CD68 and MPO; B: Damage score was evaluated by semi-quantitative analysis. C: CD68 and MPO expression were assessed by semi-quantitative analysis in SAP; *** P < 0.001 (SAP vs. Sham); #P < 0.05 (SAP vs. DCQD); ^P < 0.05 (EX527vs. DCQD); &P < 0.05 (r-HMGB1vs. DCQD); ^P <0.05 (anti-IL-23p19 vs SAP); #P <0.05 (anti-IL-17A vs SAP);

Figure3

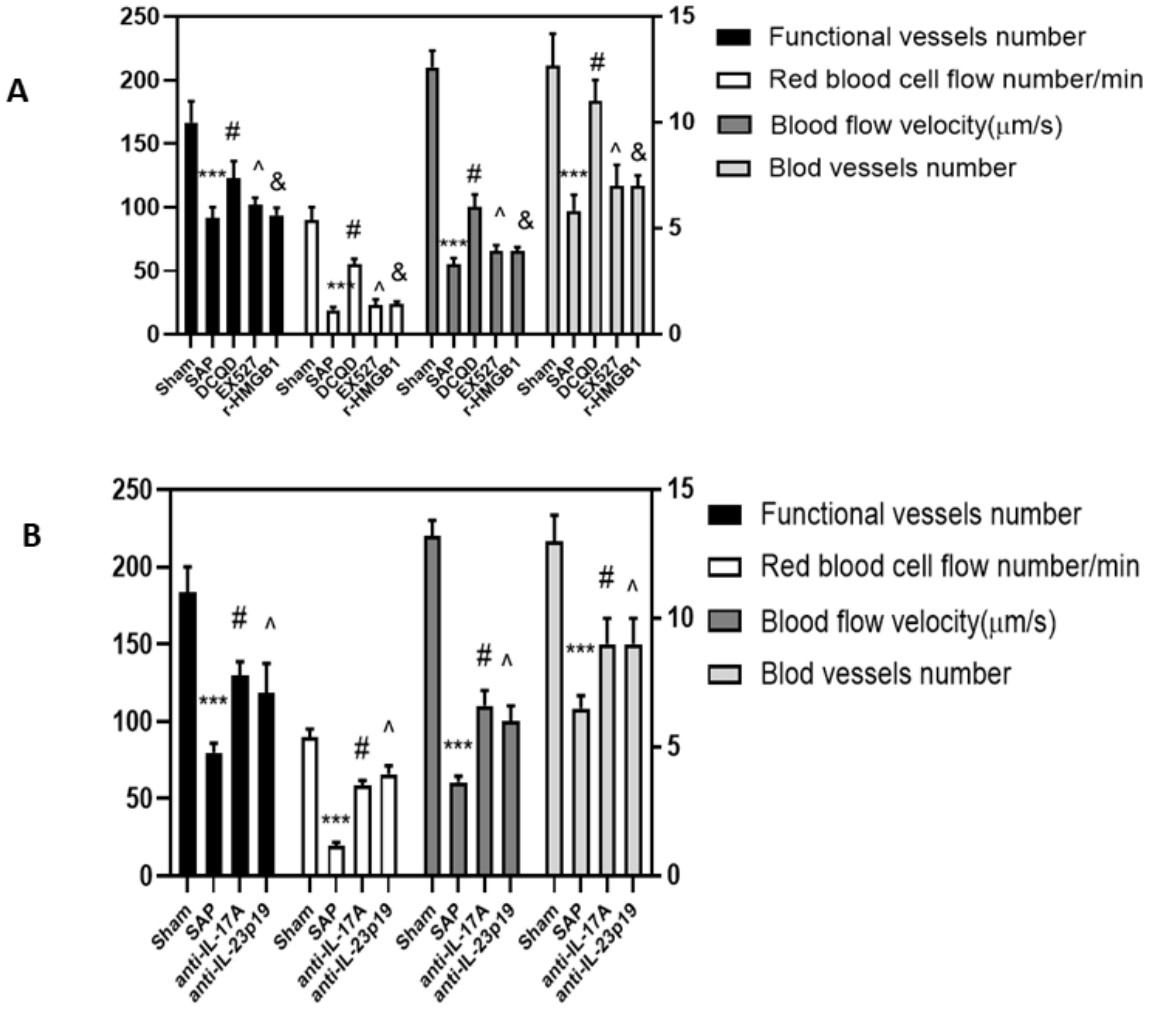

Figure 3

The effect of DCQD, EX527, anti-IL-17A, r-HMGB1 and anti-IL-23p19 on pancreatic microcirculatory function in SAP A: The effect of DCQD, EX527 and r-HMGB1 on the blood flow velocity, number of functional blood vessel, RBC flow and number of blood vessel; B: The influence of anti-IL-17A and anti-IL-23p19 on the blood flow velocity, number of functional blood vessel, RBC flow and number of blood vessel; *** P < 0.001 (SAP vs. Sham); #P < 0.05 (SAP vs. DCQD); ^P < 0.05 (EX527vs. DCQD); &P < 0.05 (r-HMGB1vs. DCQD);^P <0.05 (anti-IL-23p19 vs SAP); #P <0.05 (anti-IL-17A vs SAP);

Figure4

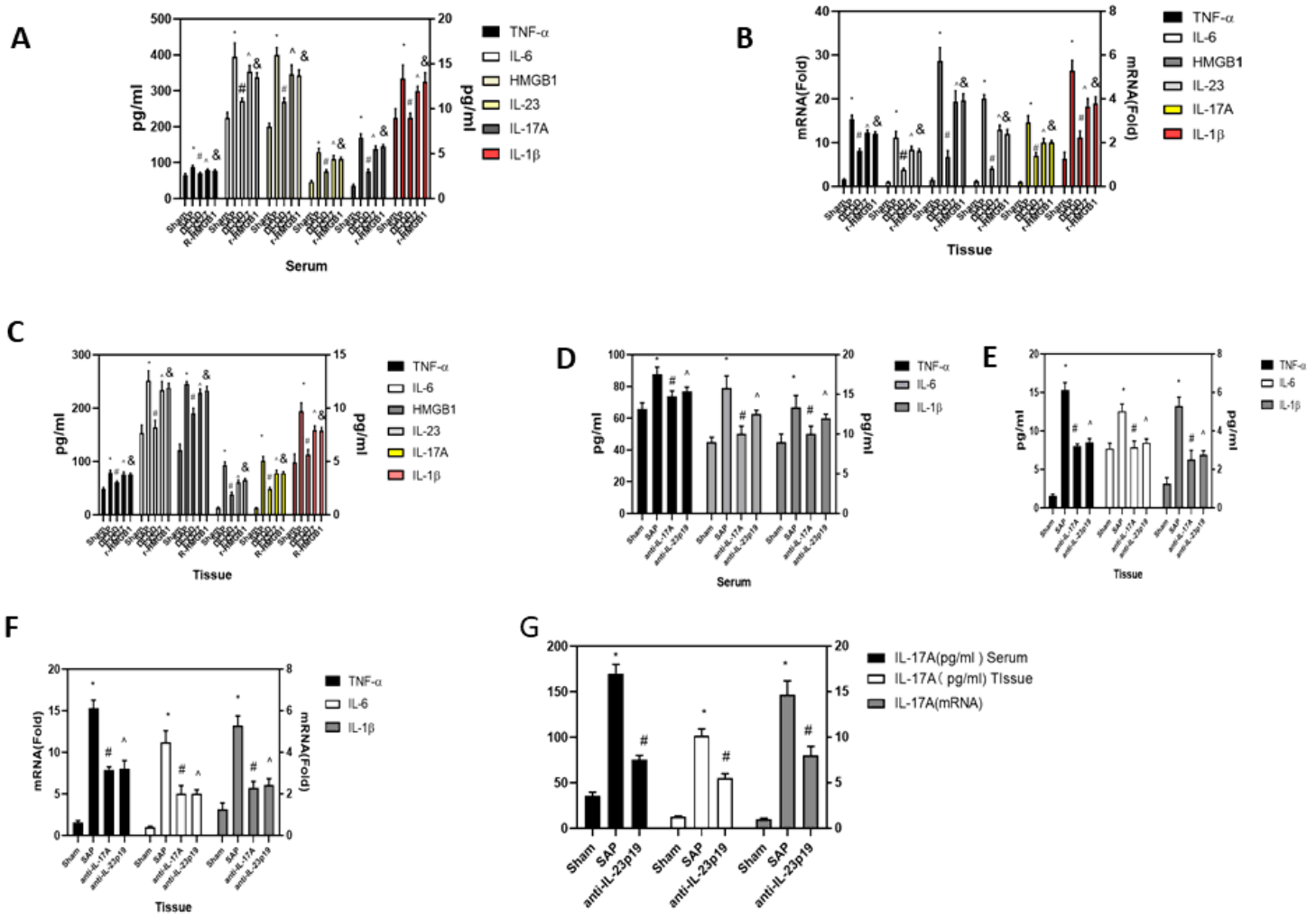

Figure 4

The influence of DCQD, EX527, anti-IL-17A, r-HMGB1 and anti-IL-23p19 on the level of IL-23, HMGB1, IL-1β, IL-17A, TNF-α and IL-6 in SAP A: ELISA was exploited to assess the influence of DCQD, EX527, and r-HMGB1 on the serum level of IL-23, HMGB1, IL-1β, IL-17A, TNF-α and IL-6 in SAP; B: RT-PCR was exploited to assay the effect of DCQD, EX527, and r-HMGB1 on the pancreatic mRNA of IL-23, HMGB1, IL-1β, IL-17A, TNF-α and IL-6 in SAP; C: ELISA was exploited to examine the effect of DCQD, EX527, and r-HMGB1 on the pancreatic level of IL-23, HMGB1, IL-1β, IL-17A, TNF-α and IL-6 in SAP;D: ELISA was exploited to text the influence of anti-IL-17A and anti-IL-23p19 on the serum level of TNF-α, IL-6 and IL-1β in SAP;E: ELISA was exploited to measure the influence of anti-IL-17A and anti-IL-23p19 on the pancreatic level of TNF-α, IL-6 and IL-1β in SAP;F: RT-PCR was exploited to measure the influence of anti-IL-17A and anti-IL-23p19 on the pancreatic mRNA of TNF-α, IL-6 and IL-1β in SAP;G: ELISA and RT-PCR were exploited to evaluate the influence of anti-IL-23p19 on the level of IL-17A in SAP; *** P < 0.001 (SAP vs. Sham); #P < 0.05 (SAP vs. DCQD); ^P < 0.05 (EX527vs. DCQD); &P < 0.05 (r-HMGB1vs. DCQD); ^P <0.05 (anti-IL-23p19 vs SAP); #P <0.05 (anti-IL-17A vs SAP);

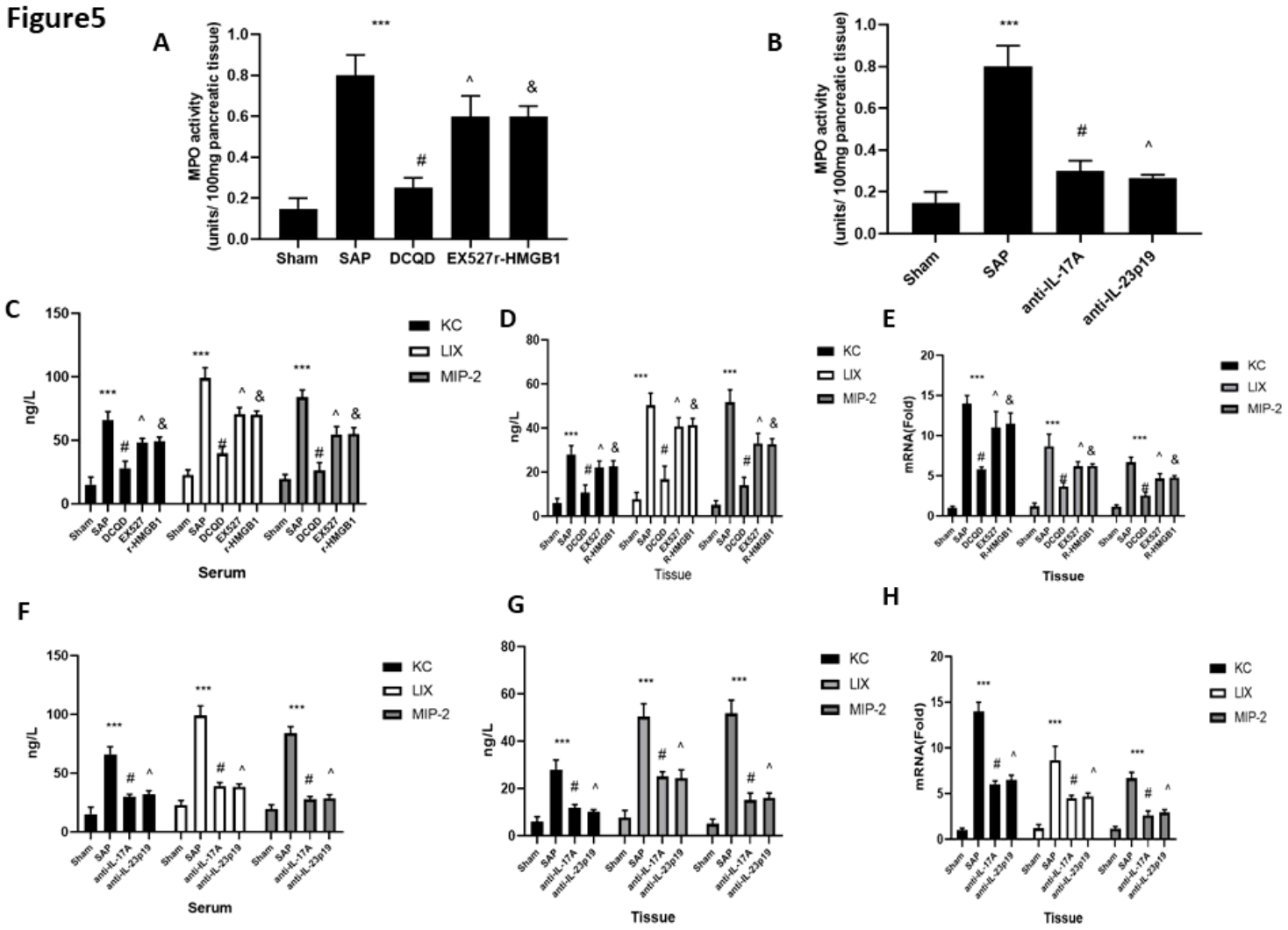

Figure 5

The influence of DCQD, EX527, anti-IL-17A, r-HMGB1, and anti-IL-23p19 on the MPO activity and MIP-2, KC and LIX expression in SAP A: ELISA was exploited to evaluate the influence of DCQD, r-HMGB1 and EX527 on the MPO activity in SAP; B: ELISA was exploited to assess the influence of anti-IL-17A and anti-IL-23p19 on the MPO activity in SAP;C ELISA was exploited to analysis the influence of DCQD, r-HMGB1 and EX527 on the serum MIP-2, KC and LIX level in SAP; D: ELISA was exploited to evaluate the influence of DCQD, r-HMGB1 and EX527 on the MIP-2, KC and LIX expression in SAP; E: RT-PCR was exploited to measure the influence of DCQD, r-HMGB1 and EX527 on the MIP-2, KC and LIX mRNA expression in SAP; F ELISA was designated to measure the influence of anti-IL-17A and anti-IL-23p19 on the MIP-2, KC and LIX serum expression in SAP; G: ELISA was designated to evaluate the influence of anti-IL-17A and anti-IL-23p19 on the MIP-2, KC and LIX pancreatic expression in SAP; E: RT-PCR was exploited to evaluate the influence of anti-IL-17A and anti-IL-23p19 on the MIP-2, KC and LIX pancreatic mRNA expression in SAP; *** P < 0.001 (SAP vs. Sham); *** P < 0.001 (SAP vs. Control); #P < 0.05 (SAP vs. DCQD); ^P < 0.05 (EX527vs. DCQD); &P < 0.05 (r-HMGB1vs. DCQD);^P <0.05 (anti-IL-23p19 vs SAP); #P <0.05 (anti-IL-17A vs SAP);

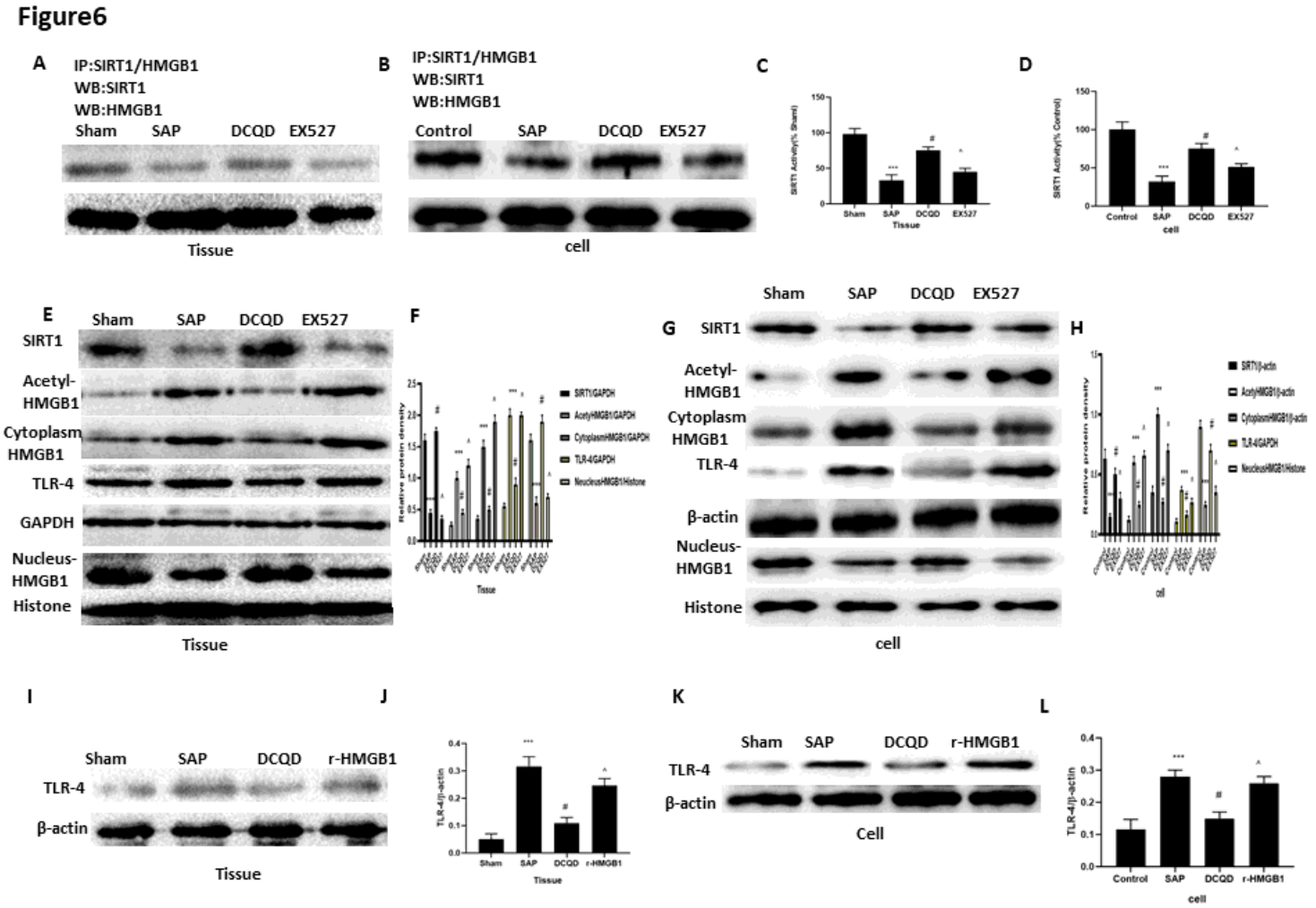

Figure 6

The influence of DCQD on the SIRT1, acety-HMGB1 and TLR-4 expression, and HMGB1 translocation, SIRT1 activity and SIRT1-HMGB1combination in pancreas in the SAP Western blotting, immunoprecipitation and ELISA were designated to evaluate the SIRT1, acety-HMGB1 and TLR-4 expression, and HMGB1 translocation, SIRT1 activity and SIRT1-HMGB1combination in vitro and vivo. A: Immunoprecipitation was exploited to assay the combination of SIRT1 and HMGB1 in the pancreas; B: Immunoprecipitation was exploited to assay the combination of SIRT1 and HMGB1 in the AR42J cell; C: The SIRT1activity in the pancreas; D: The SIRT1activity in the AR42Jcell; E: Typical result for Western blotting analysis SIRT1 in the pancreases; F:The rat SIRT1, Acety-HMGB1, HMGB1 in nuclear and cytoplasm, and TLR-4 expression was assessed by semi-quantitative analysis; G: Typical result for Western blot analysis SIRT1, Acety-HMGB1, HMGB1 in nuclear and cytoplasm, and TLR-4 on the cell; H:. The AR42J cell SIRT1 expression was assessed by semi-quantitative analysis: I: Typical results for Western blotting on TLR-4 in the SAP on rats; J: The rat TLR-4 expression was assessed by semi-quantitative analysis; K: Typical results for Western blotting on TLR-4 in the AR42J cell; L: The cell TLR-4

expression was assessed by semi-quantitative analysis; *** P < 0.001 (SAP vs. Sham); #P < 0.05 (SAP vs. DCQD); ^P < 0.05 (EX527vs. DCQD); &P < 0.05 (r-HMGB1vs. DCQD);

Figure7

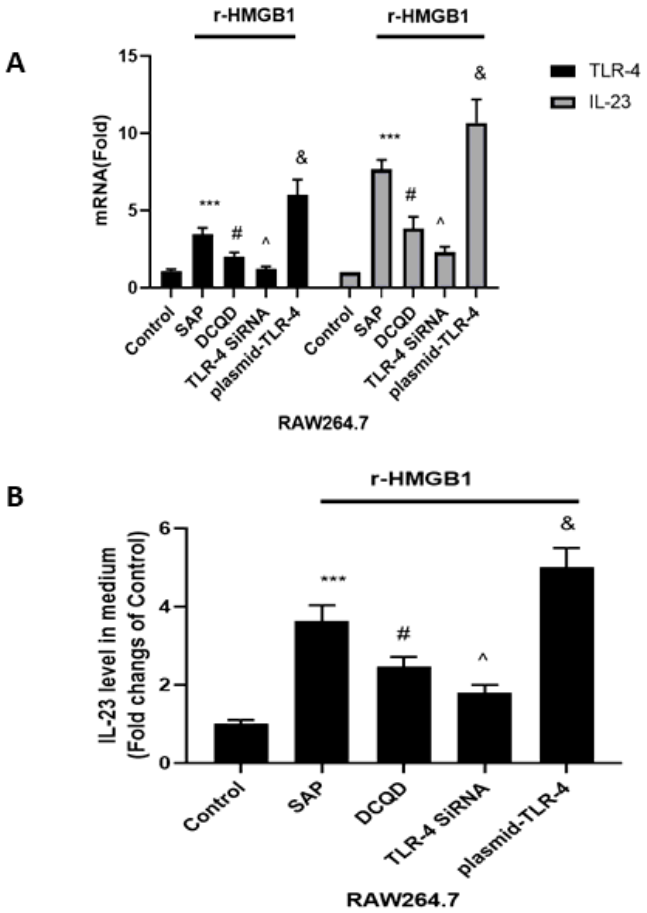

Figure 7

The influence of DCQD on the TLR-4 and IL-23 expression in SAP A: The influence of DCQD on TLR-4 and IL-23 mRNA level on RAW264.7 were detected though RT-PCR; B: ELISA was designated to evaluate the influence of r-HMGB1 on the IL-23 expression in the supernatant in SAP; ***P <0.001, (SAP vs Control); #P <0.001, (DCQD vs SAP);^P <0.05 (DCQD vs siRNA-TLR-4); &P <0.05 (DCQD vs plasmid-TLR-4);

Figure8

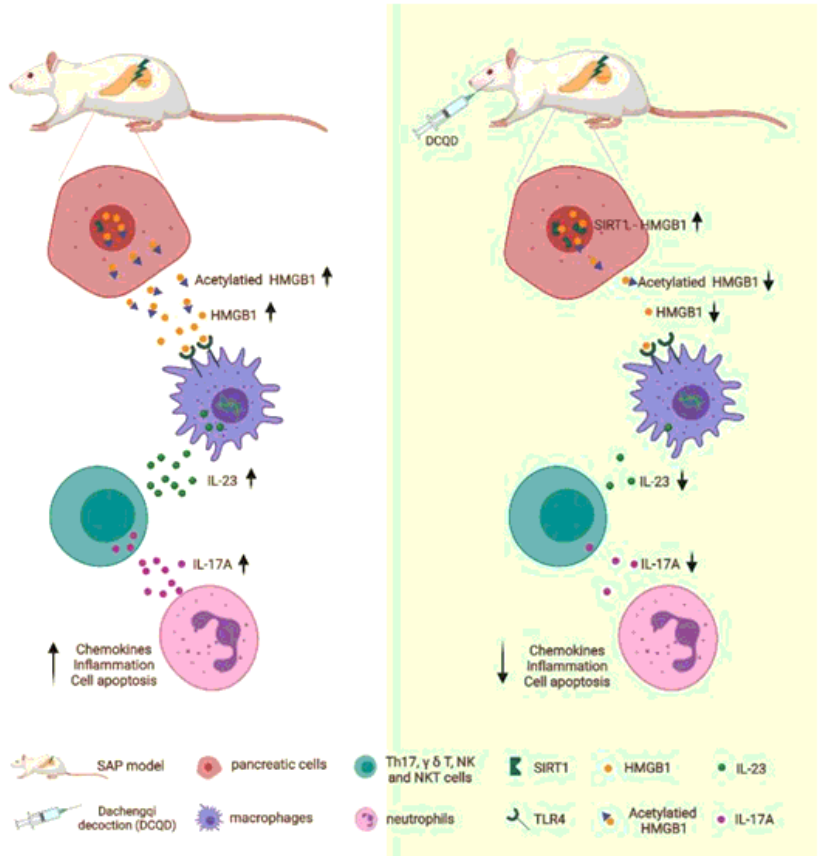

Figure 8

The signal pathway of DCQD on pancreatic microcirculatory in SAP

Supplementary Files

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