The Effects of NLRP3 Inflammasome Inhibition in Experimental Acute Pancreatitis

A Systematic Review and Meta-Analysis

Lin Gao, PhD,† Eric Chong, MSc,‡ Sayali Pendharkar, PhD,‡ Jiwon Hong, PhD,†‡ John A. Windsor, MD,†‡ Lu Ke, PhD,* Weiqin Li, MD,* and Anthony Phillips, PhD†‡*

Abstract: Acute pancreatitis (AP) is an inflammatory disease, and NLRP3 inflammasome activation is involved in the pathogenesis of AP. Previous research showed that inhibition of NLRP3 inflammasome may exert protective effects on animal models of AP and reduces disease severity. The aim of this systematic review and meta-analysis is to evaluate the effects of drug treatment of NLRP3 inflammasome on the outcomes of experimental AP. PubMed, Embase, Medline, and Web of Science databases were searched for relevant articles without language restrictions. The main outcomes for this study included local pancreatic injury, the incidence of systemic inflammatory responses, and the incidence of organ failure. Twenty-eight animal studies including 556 animals with AP were included in the meta-analysis. Compared with controls, inhibition of NLRP3 inflammasome significantly reduced the pancreatic histopathological scores, serum amylase, and lipase levels. In addition, inhibition of NLRP3 inflammasome reduced the levels of circulating inflammatory cytokines, as well as mitigating severity of AP-associated acute lung injury and acute intestinal injury. To conclude, inhibition of NLRP3 inflammasome has protective effects on AP by mitigating organ injury and systemic inflammation in animal studies, indicating that NLRP3 inflammasome holds promise as a target for specific AP therapy.

Key Words: acute pancreatitis, NLRP3 inflammasome, organ failure, pancreatic injury, systemic inflammation

(*Pancreas* 2022;51: 13–24)

Acute pancreatitis (AP) is a common inflammatory disease of the exocrine pancreas with an increasing global prevalence.[1] Although AP often presents as a mild and self-limiting condition, severe AP (SAP) is associated with significant morbidity and mortality, impaired life quality, and substantial socioeconomical burden.[2,3] Severe AP is defined by development of persistent organ failure, which is often accompanied with marked pancreatic necrosis and infection, with a morality rate as high as 30%.[4,5] Although there has been significant progress with intensive care and minimally invasive management of pancreatic necrosis, the results of drug trials have been disappointing.[6] None of the tested candidates to date have significantly reduced the incidence of organ failure and mortality.[6,7] In the majority of patients, there is a potential therapeutic window early in the disease course before the onset of persistent organ failure.[8,9] This window has yet to be exploited.

In the early stage, there are 2 critical pathophysiological events, namely, acinar cell death and systematic inflammatory response.[10] Acinar cell death initiates local pancreatic inflammation, which in turn sets up a positive autoamplification inflammatory cytokine cascade leading to the systemic inflammatory response and organ dysfunction/failure.[11,12] The cytokine "storm" is complex, with proinflammatory and anti-inflammatory components. Previous anticytokine therapies have been lopsided in addressing single proximal elements in the cytokine cascade,[13] and it is clear that a more sophisticated intervention strategy will be required to mitigate the excess inflammatory response to positively impact patient outcomes.

One of the key elements of the inflammatory response is the NLRP3 inflammasome complex.[14,15] Namely, NLRP3 (NLR family pyrin domain containing 3; previously known as NACHT, LRR, and pyrin domain containing 3) is a cytosolic sensor that recruits an adaptor molecule ASC (apoptosis-associated speck-like protein with a caspase recruitment domain), and an effector cysteine protease caspase 1, to form the macroscopic NLRP3 inflammasome complex.[14,15] Active caspase 1 has 2 major functions. It does not only process pro–interleukin (IL) 18 and IL-1β into their bioactive forms to promote inflammation but also cleaves gasdermin D, the activated fragment of which can form pores on the plasma membrane to initiate cell death and allow the release of mature IL-18 and IL-1β (see Fig. 1).[16–18] This highlights that NLRP3 inflammasome functions as the key link between cell death and systemic inflammation, which may also be a potentially therapeutic target in various inflammatory diseases. Studies have shown that NLRP3 inflammasome is closely related to the pathogenesis of some diseases, including myocardial infarction, cerebral ischemia, neurodegenerative diseases, type 2 diabetes, and septic shock.[14,19,20] There have also been a number of studies targeting the NLRP3 inflammasome in animal models of AP.[21] The aim of this systematic review and meta-analysis was to evaluate the effects of inhibiting NLRP3 inflammasome on the main outcomes from experimental AP.

MATERIALS AND METHODS

This systematic review was prepared using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidance for literature review, extraction of data, and reporting of results.[22]

From the *Center of Severe Acute Pancreatitis, Department of Critical Care Medicine, Jinling Hospital, Medical School of Nanjing University, Nanjing, Jiangsu, China; †Applied Surgery and Metabolism Laboratory, School of Biological Sciences, and ‡Surgical and Translational Research Centre, Faculty of Medical and Health Sciences, School of Medicine, University of Auckland, Auckland, New Zealand.

Received for publication December 8, 2020; accepted December 16, 2021.

Address correspondence to: Lu Ke, PhD, Center of Severe Acute Pancreatitis, Department of Critical Care Medicine, Jinling Hospital, Medical School of Nanjing University, 305 Zhongshan East Rd, Xuanwu District, Nanjing 210023, Jiangsu, China (e-mail: ctgkelu@nju.edu.cn).

The authors declare that there is no conflict of interest regarding the research, authorship, and/or publication of this article. The authors received no financial support for the research, authorship, and/or publication of this article.

Supplemental digital contents are available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pancreasjournal.com).

DOI: 10.1097/MPA.0000000000001971

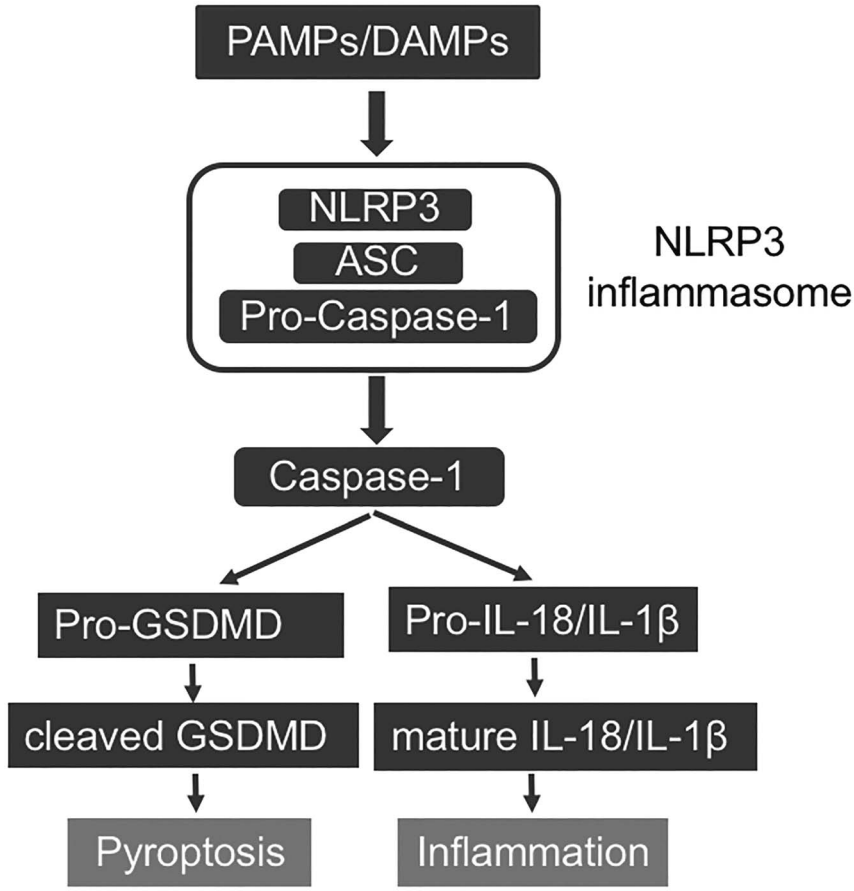

FIGURE 1. The canonical NLRP3 inflammasome activation pathway. Danger signals, such as pathogen-associated molecular patterns/damage-associated molecular patterns, induce the activation of NLRP3 inflammasome, which consists of cytosolic sensor NLRP3, adaptor molecule ASC, and an effector cysteine protease caspase 1. Active caspase 1 has 2 major functions. It processes pro-IL-18 and pro-IL-1β into their respective bioactive forms to promote inflammation, and cleaves gasdermin D, the activated fragment of which can form pores on the plasma membranes to initiate cell death and allow the release of mature IL-18 and IL-1β.

Search Strategy

Two investigators (L.G. and E.C.) independently searched PubMed, Embase, Medline, and Web of Science from their inception to May 10, 2020 for relevant articles without language restrictions. The reference lists of the included studies were checked for additional relevant studies. The following keywords or MeSH headings were used: "NLRP3" OR "NLRP3 inflammasome" AND "acute pancreatitis."

Study Selection Criteria

The following preclinical studies were included:

1. Study subjects: animal models with induced AP;
2. Intervention: drugs or gene modification inhibiting NLRP3 inflammasome activation;
3. Comparison: no treatment;
4. Outcomes: local pancreatic injury, incidence of systemic inflammatory responses, and incidence of organ failure; and
5. Study design: parallel group animal studies.

Studies lacking main outcomes of interest, experiments conducted in animal models with induced chronic pancreatitis, experiments conducted on in vitro AP models, and studies not focused on NLRP3 inflammasome were excluded.

Data Extraction

Data from included studies were extracted by 2 independent authors (L.G. and E.C.), and discrepancies were resolved through discussion until consensus was reached. The data extracted from selected animal studies were as follows: first author's name, year of publication, animal models used, details of intervention and control, main outcomes, and key findings. When numerical data were only presented in figures, Engauge Digitizer (version 12.1; developed and maintained by Mark Mitchell, Torrance, Calif) was used to extract and digitize the data points.[23]

Outcomes

The main outcomes of interest for this study included local pancreatic injury (histopathological scores, serum amylase, and lipase levels), the incidence of systemic inflammatory responses based on circulating inflammatory cytokine levels (including IL-1β, IL-6, and tumor necrosis factor [TNF] α), and the incidence of organ dysfunction/failure, including AP-associated acute lung injury (histological scores) and acute intestinal injury (colon crypt length was used as a proxy for severity of acute intestinal injury, and the shorter the colon crypt length, the severer the intestinal injury).

Assessment of Risk of Bias

For included studies, study quality and risk of bias assessment were based on the SYRCLE (Systematic Review Center for Laboratory animal Experimentation) grading system,[24] which is an adapted version of the Cochrane risk of bias tool.[25] Each item was assigned a low, unclear, or high risk of bias.

Statistical Analysis

The results were presented as forest plots through the standardized mean difference (SMD) with 95% confidence interval (CI) for continuous data with an inverse variance method. The I^2 statistic was used to assess statistical heterogeneity among the studies. Values of I^2 greater than 50% indicated moderate heterogeneity, and over 75% indicated a high level of heterogeneity. If heterogeneity was observed ($I^2 \geq 50\%$), the random-effects model was applied; otherwise, a fixed-effects model was used. Publication bias was assessed by funnel plot and Egger regression if sufficient data were available. All analyses except publication bias evaluation were performed by Review Manager 5.3 software (The Nordic Cochrane Center, Copenhagen, Denmark). Publication bias evaluation, including funnel plot, Egger test, and Begg test, was performed by using Stata software version 15.0 (StataCorp, College Station, Tex). A 2-sided P value <0.05 was considered statistically significant.

RESULTS

Study Selection

The database search identified 453 records. After duplicates removal, the remaining 278 records were screened by their titles and abstracts. A total of 233 nonrelevant studies were excluded, and the remaining 45 full-text articles were assessed for eligibility. Notable exclusions were studies that did not use AP animal models (n = 6),[26–31] or used an in vitro AP model (n = 1),[32] or had interventions that did not target NLRP3 inflammasome inhibition (n = 7).[33–39] Two review/commentary articles were also excluded,[40,41] and 1 study did not report the main outcomes of interest.[42] This left 28 studies that were included in the systematic review and meta-analysis (Fig. 2).[12,43–69]

Study Characteristics

Table 1 shows the details of the 28 animal studies, which included 35 experiments involving 280 control animals and 276 AP-induced animals with treatment. Seventeen of the included 28 studies were published in the past 3 years with the oldest study published in 2011.[12] Rats (*Rattus norvegicus*) were used in 4 studies, and mice (*Mus musculus*) in 24 studies. The animal models of AP used in these experiments included cerulein-induced AP (n = 17), cerulein plus lipopolysaccharide (LPS)–induced SAP (n = 9), and retrograde infusion of sodium taurocholate–induced SAP (n = 7). NLRP3 inflammasome inhibition was achieved by drug administration in 30 experiments and gene modified animals (mainly with *NLRP3* gene knockout mice) in 5 experiments.[12,43,50,52,64] Only 3 studies used a specific NLRP3 inflammasome inhibitor (glyburide, INF-39, and MCC950),[43,50,63] and most of the others adopted drugs that inhibited the activation of NLRP3 inflammasome indirectly.

Risk of Bias

Randomization, allocation concealment, blinding, sample size calculations, and other items in SYRCLE's risk of bias tool were not reported. Hence, it was impossible to assess risk of bias in these animal studies. The risk of bias was unclear or high in all studies, and study quality was therefore judged to be low.

Effect of NLRP3 Inflammasome Inhibition on Local Pancreatic Injury

Twenty studies that included 25 experiments reported the effects of NLRP3 inflammasome inhibition on pancreatic injury in animal models with AP, and the meta-analysis of them was undertaken. The results showed that inhibiting NLRP3 inflammasome was associated with a reduced histopathological score of

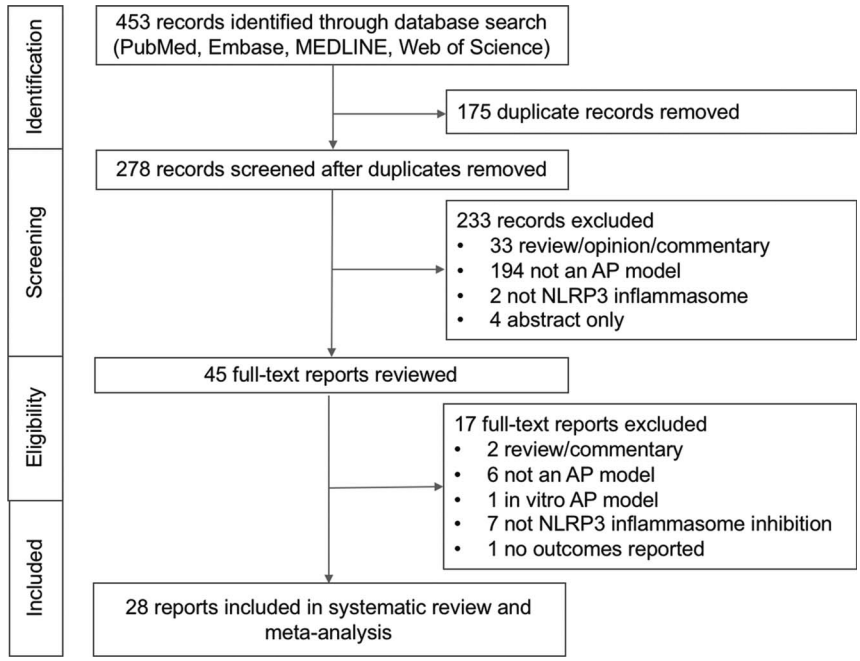

FIGURE 2. Flow diagram that summarizes the results of the literature search.

TABLE 1. Characteristics of Animal Studies Included in the Systematic Review and Meta-Analysis

Study, Year	Animal Model	Interventions	Drug Properties	Main Outcomes	Key Findings
Hoque et al, 2011[12]	Male C57BL/6 mice with CER-induced AP model	WT and *NLRP3*$^{-/-}$ mice	NA	Histology and histology scores (pancreas)	Deletion of inflammasome component reduces the severity of AP
Farooq et al, 2014[67]	Male C57BL/6 mice with CER + LPS–induced SAP model	Aspartate (300 mM, 30 μL/g) was injected intraperitoneally at first and third dose of CER	An *N*-methyl-D-aspartate receptor activator	Histology (pancreas); serum amylase levels	Aspartate supplementation reduces pancreatic inflammasome levels and protects from CER-induced pancreatitis
Hoque et al, 2014[56]	C57BL/6N male mice with CER + LPS–induced SAP model	Subcutaneous 150 mmol/L sodium lactate at 30 mL/g with the third dose of CER	A carboxylic acid	Histology (liver/pancreas); serum ALT and amylase levels	In macrophages and monocytes, increasing concentrations of lactate reduced TLR4-mediated induction of NLRP3 inflammasome; in vivo, lactate reduced the severity of AP and acute liver injury
Ren et al, 2014[48]	Male Balb/c mice with CER-induced AP model	An intraperitoneal injection of hydrogen-rich saline at 8 mL/kg, every 20 min for 3 times	An antioxidant	Histology (pancreas); serum amylase and lipase levels; serum TNF-α and IL-1β levels	Hydrogen-rich saline inhibits NLRP3 inflammasome activation in pancreatic tissues in AP
York et al, 2014[63]	Obese ob/ob mice with CER-induced AP model	Intraperitoneal injections of glyburide (500 mg/kg) at −13, −1, and 9 h relative to induction of AP	Sulfonylurea drug; an NLRP3 inflammasome inhibitor	Histology (pancreas); serum amylase and lipase, IL-6, IL-1β levels	Glyburide reduces disease severity in obese mice with CER-induced AP
Dong et al, 2016[54]	BALB/c mice with CER-induced AP model	Mice were treated with sulforaphane (5 mg/kg) once a day for 3 consecutive days before the induction of AP	A natural organosulfur antioxidant	Histology (pancreas); serum amylase levels; pancreatic MPO levels	Sulforaphane modulates Nrf2-mediated oxidative stress and NLRP3/NF-κB inflammatory pathways in acinar cells, thereby protecting against AP
He et al, 2016[55]	WT C57BL/6 mice with retrograde infusion of 2% sodium taurocholate–induced SAP model	Human bone marrow–derived mesenchymal stromal cells administration	Stem/progenitor cells with actions of migration, engraftment, and differentiation to repair tissues	Histology (pancreas); serum amylase and lipase; pancreatic MPO levels; serum IL-6, TNF-α, and IL-1β levels	The protective effects of bone marrow–derived mesenchymal stromal cells on SAP via inhibition of NLRP3 inflammasome and NF-κB signaling pathways
Kanak et al, 2017[66]	C57BL/6 mice with CER-induced AP model	Withaferin A (1.25 mg/kg) was administered 1 h before the first CER injection	A small molecule inhibitor of NF-κB	Histology (pancreas); serum amylase levels	Withaferin A reduces the severity of AP and abolishes immune cell infiltration
Li et al, 2018[68]	Male ICR mice with CER-induced AP model; male ICR mice with CER + LPS–induced SAP model	Dexmedetomidine at 20 μg/kg was injected intravenously at 0, 2, 4, 6, and 8 h after first CER injection	A highly selective α2-adrenergic receptor agonist	Histology (pancreas/lungs); serum amylase and lipase levels; serum TNF-α, IL-6, and IL-1β levels	Dexmedetomidine attenuates pancreatic injury and inflammatory response in mice with pancreatitis possibly by reducing NLRP3 activation and upregulating norepinephrine transporter expression
Lu et al, 2017[59]	Female ICR mice with CER + LPS–induced SAP model	Indomethacin (8 mg/kg) was injected 0 h and 12 h after induction of AP	A cyclo-oxygen-ase-2 inhibitor	Histology (pancreas); serum amylase and lipase levels; serum TNF-α and IL-1β levels	Indomethacin could protect pancreatic acinar cell from injury and alleviate the severity of SAP by inhabiting NLRP3 inflammasome pathway

Shen et al, 2017[51]	Male C57BL/6 mice with CER-induced AP model	β-Lapachone (40 mg/kg) orally injected at 24 and 3 h before induction of AP	A quinone-containing natural compound with the actions of increasing cellular NAD^+ levels by NQO1 enzymatic action	Histology (pancreas); pancreatic MPO levels; serum amylase and lipase levels	β-Lapachone suppressed CER-induced AP with down-regulating toll-like receptor 4-mediated inflammasome signaling, and thereby reducing the inflammatory responses and pancreatic cell death
Fu et al, 2018[43]	C57BL/6 mice with CER + LPS–induced SAP model	WT and *NLRP3*$^{-/-}$ mice; NLRP3 inhibitor INF-39 administration (50 mg/kg, 30 min after the first injection of CER)	An NLRP3 inflammasome inhibitor	Histology (pancreas/lung); serum amylase and lipase levels; serum/peritoneal lavage fluid IL-6, TNF-α, and IL-1β levels	SAP and SAP-induced acute lung injury were alleviated by NLRP3 deficiency in mice, and NLRP3 inhibitor could mitigate SAP-associated inflammation and acute lung injury
Li et al, 2018[58]	Male ICR mice with CER-induced AP model	INT-777 (1 mg per mice) was given 1 h before the induction of AP	A bile acid receptor agonist	Histology (pancreas); serum amylase and lipase levels; serum IL-6, TNF-α, and IL-1β levels	INT-777 could protect pancreatic acinar cell against necrosis and reduce the severity of AP, which may be mediated by inhibiting NLRP3 inflammasome pathway
Li et al, 2018[69]	Male ICR mice with CER-induced AP model; male ICR mice with L-arginine–induced SAP model	Naringenin (100 mg/kg) injected intraperitoneally after AP induction immediately	A type of flavonoid, the predominant flavanone in grapefruit	Histology (pancreas/lungs); serum amylase and lipase levels; serum TNF-α, IL-6, and IL-1β levels	Naringenin exerts protective effects on AP by inhibiting oxidative stress and inflammatory response via the inactivation of NLRP3 inflammasome and upregulation of Nrf2/HO 1 expression
Ren et al, 2018[49]	Female Balb/c mice with CER-induced AP model	Danshensu derivative DSC was administered intraperitoneally at a dose of 100 mg/kg before or 1 h after the induction of AP	A derivative of danshen, which is a natural Chinese herb	Histology (pancreas); serum amylase and lipase levels; serum IL-6, TNF-α, and IL-10 levels	DSC alleviates pancreatic inflammation and damage in AP by inhibiting the activation of NF-κB, STAT3, and NLRP3 inflammasome and modulating immune cell responses
Xiong et al, 2018[61]	SD rats with retrograde infusion of 3.5% sodium taurocholate–induced SAP model	Intragastric administration of 72 mg/kg free total rhubarb anthraquinones immediately and 12 h after induction of SAP	A derivative of rhubarb, which is a traditional Chinese medicine	Histology (intestinal tissues); levels of endotoxin, TNF-α, and IL-1β in the blood or intestinal tissues	Rhubarb anthraquinones could protect intestinal injury and improve intestinal mucosal barrier function; it significantly decreased the expressions of NLRP3 and caspase 1 in the intestinal tissues in SAP rats
Yu et al, 2019[64]	C57BL/6 mice with CER + LPS–induced SAP model	WT and humanized transgenic surfactant protein D mice	Innate immune molecule surfactant protein D, a member of C-type lectin family	Histology (pancreas/lung); serum amylase levels; serum IL-1β, IL-6 levels	Surfactant protein D exerts protective effects against acute lung injury via suppressing NLRP3 inflammasome and NF-κB activation in experimental SAP
Hou et al, 2019[57]	Male C57BL/6 mice with CER + LPS–induced SAP model	Iguratimod (5 mg/kg) was injected intraperitoneally after induction of AP	A selective inhibitor of cyclooxygenase 2	Histology (pancreas); MPO expression of pancreas; serum amylase and lipase levels; inflammatory factors in blood and pancreas	Iguratimod plays a protective role in experimental SAP mice model by inhibiting NLRP3 inflammasome pathways
Jin et al, 2019[45]	Male Wistar rats with retrograde infusion of 5% sodium taurocholate–induced SAP model	Apocynin was injected (50 mg/kg) 30 min before the induction of SAP model	A nicotinamide adenine dinucleotide phosphate oxidase inhibitor	Histology (pancreas/lung); serum amylase and lipase levels; serum inflammatory factors levels	Apocynin has anti-inflammatory effects by suppressing NLRP3 inflammasome activation and NF-κB signaling in SAP and SAP-induced lung injury

(Continued on next page)

TABLE 1. *(Continued)*

Study, Year	Animal Model	Interventions	Drug Properties	Main Outcomes	Key Findings
Kim et al, 2019[46]	C57BL/6 mice with CER-induced AP model	Fraxinellone (5 mg/kg) or parthenolide (25 mg/kg) was intraperitoneally administered 1 h before induction of AP	A limonoid component isolated from *Dictamnus dasycarpus*	Histology (pancreas); MPO expression of pancreas; serum amylase and lipase levels	Fraxinellone/parthenolide protected against AP by inhibiting NLRP3 signaling pathways
Pan et al, 2019[65]	BALB/c mice with CER-induced AP model; C57BL/6J mice with CER + LPS–induced SAP model	*Clostridium butyricum* strain (5.7×10^9 colony-forming unit/kg per day) by gavage once a day for 21 consecutive days before induction of AP	Short chain fatty acids producing probiotics	Histology (pancreas/colon); serum and colonic inflammatory factors levels	*C. butyricum* inhibited pancreatic NLRP3 inflammasome activation as well as proinflammatory signaling pathways. *C. butyricum* strains attenuated AP-associated intestinal inflammation and barrier dysfunction
Pan et al, 2019[47]	BALB/c mice with CER-induced AP model	Sodium butyrate was administered intragastrically for 7 d before induction of AP	A short-chain fatty acid produced from the fermentation of dietary fibers by intestinal microbiota	Histology (pancreas/colon); serum amylase and lipase levels; inflammatory factors levels in pancreatic and colonic tissues	Butyrate is effective to ameliorate AP and associated intestinal injury via suppressing NLRP3 inflammasome activation and modulating immune cell infiltration in pancreas
Xia et al, 2019[60]	SD rats with retrograde infusion of 5% sodium taurocholate–induced SAP model	Three-time intragastric administration of 6 mg/mL emodin after induction of SAP	One main active component of rhubarb, which is a traditional Chinese medicine	Histology (pancreas); serum amylase and lipase levels; serum TNF-α and IL-18 levels	Emodin plays its protective role on SAP against oxidative stress and inflammasome signals
Zhang et al, 2019[53]	SD rats with retrograde infusion of 5% sodium taurocholate–induced SAP model	Emodin was intragastrically applied to the rats immediately and at 6 h after induction of SAP	A main active component of rhubarb	Histology (pancreas); MPO expression of pancreas; serum amylase and lipase levels; serum IL-1β and IL-18 levels	Emodin has a protective effect on AP by inhibiting P2X7/NLRP3 signaling pathways
Jia et al, 2020[44]	Female BALB/c mice with CER-induced AP model	Antibiotic combination (vancomycin, neomycin, and polymyxin b) administration for 8 d	Antibiotic combination	Histology (pancreas/colon); inflammatory factors levels in pancreatic and colonic tissues	Combinatory antibiotics therapy inhibited the translocation of gut bacteria to pancreas by inhibiting the pancreatic NLRP3 pathway, and inhibiting intestinal-pancreatic inflammatory responses
Sendler et al, 2020[50]	C57BL/6 mice with partial pancreatic duct ligation and a single injection of CER (50 mg/kg) 2 d after surgery-induced SAP	WT and *NLRP3*$^{-/-}$ mice; MCC950 administration (2 injections of 50 mg/kg body weight 24 h and 48 h after surgical duct ligation)	A specific NLRP3 inflammasome inhibitor	Histology (pancreas); serum amylase levels	In pancreatitis, significantly decreased disease severity was shown in *NLRP3* gene knockout mice or MCC950 treatment
Wu et al, 2020[52]	Male C57BL/6 mice with L-arginine injection–induced SAP model	WT and *NLRP3*$^{-/-}$ mice	NA	Histology of lungs; serum amylase and lipase levels	Activation of NLRP3 inflammasome contributed to AP-induced acute lung injury
Yang et al, 2020[62]	Male ICR mice with CER-induced AP model	Cordycepin was administered intragastrically 2 h before induction of AP	A derivative from *Cordyceps militaris*, which is a traditional Chinese medicine	Histology (pancreas); serum amylase and lipase levels; serum inflammatory factors levels	Cordycepin protects mice from pancreatic inflammatory process and damage by suppressed NF-κB and NLRP3 inflammasome activation, which probably contributed to the potential therapy for AP

Note: The animal numbers in each group in studies Xia et al, 2019[60]; Farooq et al, 2014[67]; Wu et al, 2020[52]; and Dong et al, 2016[54] were not reported, and we took 6 as a default value.

CER indicates cerulein; AP, acute pancreatitis; ICR, Institute of Cancer Research; WT, wild-type; NA, not applicable; SAP, severe acute pancreatitis; ALT, alanine transaminase; MPO, myeloperoxidase; NF-κB, nuclear factor κB; SD, Sprague-Dawley.

pancreatic injury (SMD, −3.68; 95% CI, −4.58 to −2.78; $P < 0.001$) (Fig. 3). The study heterogeneity was high (I^2 = 85%), and a random-effects model was used for this analysis. The overall effect of NLRP3 inflammasome inhibition on pancreatic injury was highly significant ($P < 0.001$), and it did not differ statistically (P = 0.79) among the different AP animal models (cerulein-induced AP model [SMD, −3.34; 95% CI, −4.55 to −2.14; $P < 0.001$] vs cerulein + LPS–induced SAP model [SMD, −4.07; 95% CI, −5.87 to −2.26; $P < 0.001$] vs sodium taurocholate–induced SAP model [SMD, −3.83; 95% CI, −6.07 to −1.59; $P < 0.001$]), and there was moderate or high heterogeneity across each group of studies (Supplemental Fig. 1, http://links.lww.com/MPA/A920). Similarly, the effects of NLRP3 inflammasome inhibition on pancreatic injury were compared among gene modified NLRP3 inhibition, specific NLRP3 inhibitor use, and nonspecific NLRP3 inhibitor use. The overall effect was highly significant ($P \leq 0.001$), and it did not differ statistically (P = 0.46) among the different intervention strategies (gene modified NLRP3 inhibition [SMD, −2.45; 95% CI, −5.50 to 0.60; P = 0.12] vs specific NLRP3 inhibitor [SMD, −2.76; 95% CI, −5.59 to 0.08; P = 0.06] vs nonspecific NLRP3 inhibitor [SMD, −4.08; 95% CI, −5.12 to −3.04; $P \leq 0.001$]) (Supplemental Fig. 2, http://links.lww.com/MPA/A920). A symmetry funnel plot (Supplemental Fig. 3, http://links.lww.com/MPA/A920), Begg test (P = 0.907), and Egger test (P = 0.455) indicated the absence of publication bias.

There were 25 studies (including 33 experiments) that reported serum amylase levels and 19 studies (including 26 experiments) that reported serum lipase levels. The meta-analysis showed that NLRP3 inflammasome inhibition significantly reduced the serum amylase (SMD, −1.91; 95% CI, −2.36 to −1.45; $P < 0.001$) (Fig. 4) and lipase levels (SMD, −1.72; 95% CI, −2.17 to −1.28; $P < 0.001$) (Supplemental Fig. 4, http://links.lww.com/MPA/A920) in AP animal models. There was high heterogeneity across the included studies.

Effect of NLRP3 Inflammasome Inhibition on Systemic Inflammation

There were 15 studies (including 18 experiments) that evaluated the effects of NLRP3 inflammasome on serum IL-1β levels, and the meta-analysis showed that NLRP3 inflammasome inhibition was associated with a significant reduction in serum IL-1β levels in animal models with AP (SMD, −2.82; 95% CI, −3.66 to −1.98; $P < 0.001$) (Fig. 5). Similarly, the meta-analysis of 11 studies (including 16 experiments) found a significant decrease in serum IL-6 levels (SMD, −1.76; 95% CI, −2.39 to −1.13; $P < 0.001$) (Supplemental Fig. 5, http://links.lww.com/MPA/A920), and the meta-analysis of 13 studies (including 17 experiments) showed significantly reduced serum TNF-α levels (SMD, −2.16; 95% CI, −2.65 to −1.68; $P < 0.001$) (Supplemental Fig. 6, http://links.lww.com/MPA/A920) in animals with NLRP3 inflammasome inhibition, respectively. Collectively, these meta-analysis results showed a significant association between NLRP3 inflammasome inhibition and mitigated systemic inflammation.

Effect of NLRP3 Inflammasome Inhibition on Organ Failure

There were 5 studies[45,52,64,68,69] that reported histopathological scores of acute lung injury and 3 studies[44,47,65] that reported colon crypt length. The meta-analysis showed that NLRP3 inflammasome inhibition significantly decreased acute lung injury in animal models with AP (SMD, −2.74; 95% CI, −4.92 to −0.55; P = 0.01) (Fig. 6). In animals with acute intestinal injury, inhibiting NLRP3 inflammasome was associated with a significantly longer colon crypt length (SMD, 1.79; 95% CI, 1.04–2.54; $P < 0.001$)

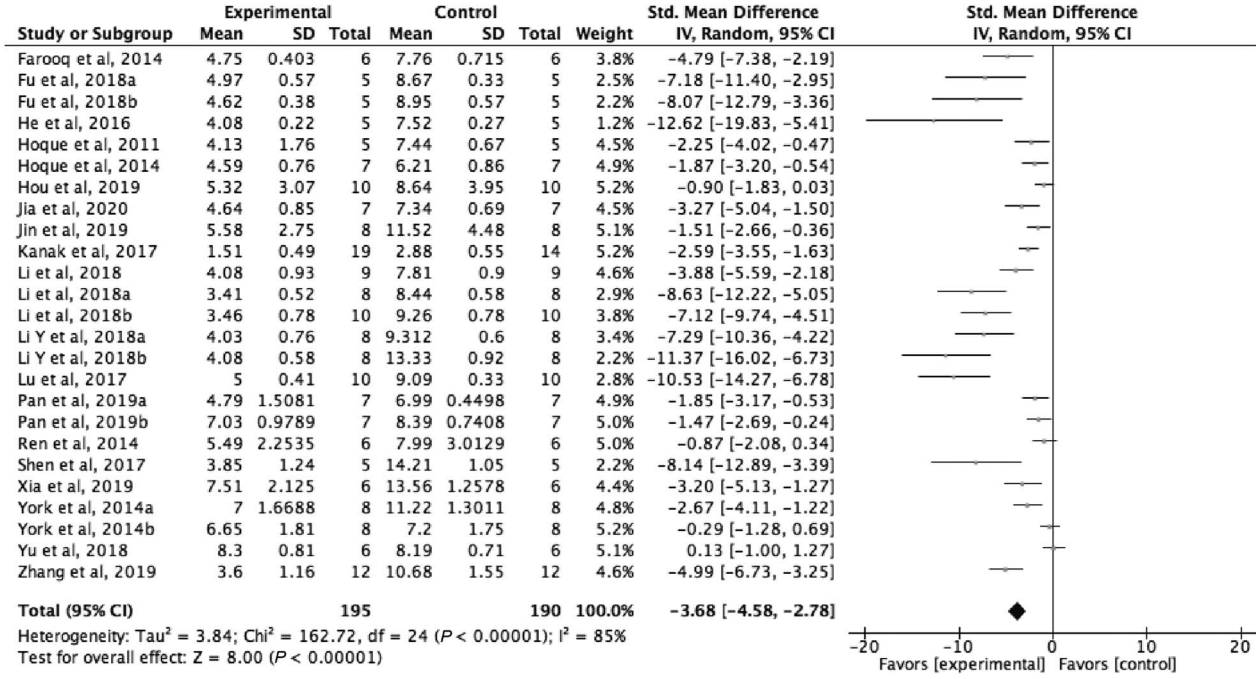

FIGURE 3. Forest plot of the effect of NLRP3 inflammasome inhibition on histopathological score of pancreatic injury in animal models with AP. There were 2 experiments (named "a" and "b," respectively) included in the meta-analysis, in the studies by Fu et al, 2018[43]; Li et al, 2018[58]; Li et al, 2018[68,69]; Pan et al, 2019[47,65]; and York et al, 2014.[63] To distinguish them, the suffixes "a" and "b" were used in these studies; IV indicates inverse variance; Tau^2, τ^2 statistic; Chi^2, χ^2 statistic; *df*, degrees of freedom; I^2, I^2 heterogeneity statistic; *Z*, *Z* statistic.

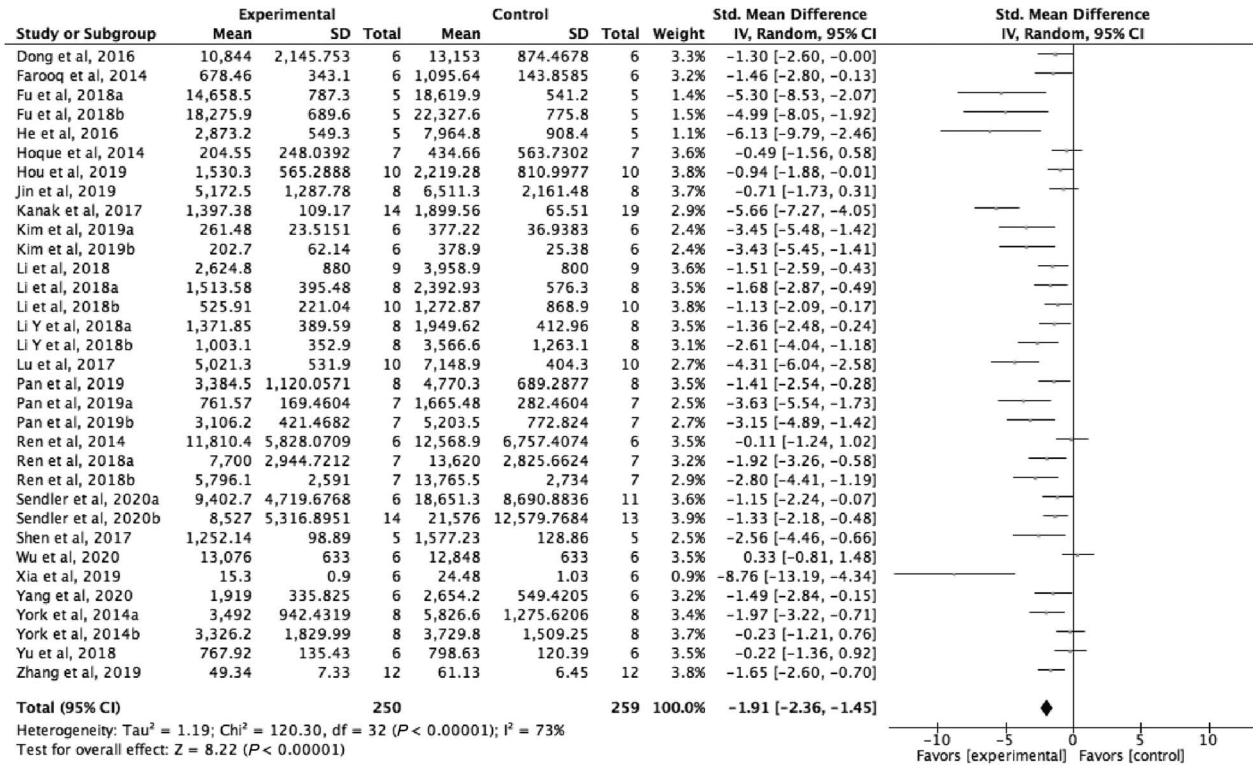

FIGURE 4. Forest plot of the effect of NLRP3 inflammasome inhibition on serum amylase levels in animal models with AP. There were 2 experiments (named "a" and "b," respectively) included in the meta-analysis, in the studies by Fu et al, 2018[43]; Kim et al, 2019[46]; Li et al, 2018[58]; Li et al, 2018[68,69]; Pan et al, 2019[47,65]; Ren et al, 2018[49]; Sendler et al, 2020[50]; and York et al, 2014.[63] To distinguish them, the suffixes "a" and "b" were used in these studies. IV indicates inverse variance; Tau^2, τ^2 statistic; Chi^2, χ^2 statistic; *df*, degrees of freedom; I^2, I^2 heterogeneity statistic; *Z*, *Z* statistic.

(Supplemental Fig. 7, http://links.lww.com/MPA/A920), indicating less severe intestinal injury.

DISCUSSION

This systematic review of 28 studies assessed the effects of NLRP3 inflammasome inhibition on local pancreatic injury, systemic inflammation, and organ failure in animal models of AP. The meta-analysis confirms that inhibiting NLRP3 inflammasome is protective in AP, demonstrating reduced pancreatic, lung, and intestinal injury, and reduced serum amylase and lipase levels, as well as serum levels of inflammatory cytokines IL-1β, IL-6, and TNF-α. The effects of NLRP3 inflammasome inhibition did not

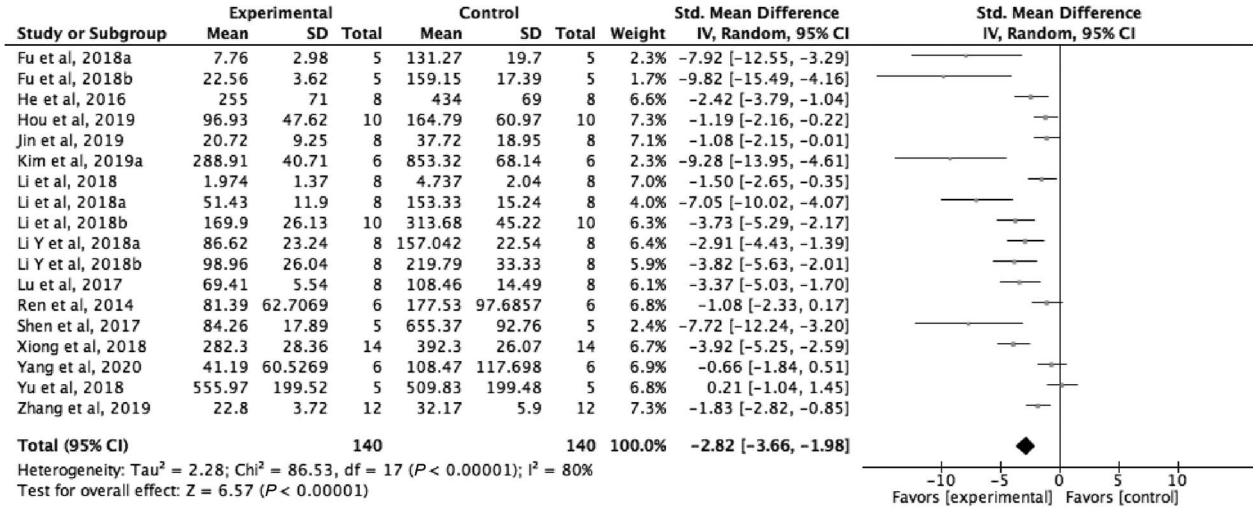

FIGURE 5. Forest plot of the effect of NLRP3 inflammasome inhibition on serum IL-1β levels in animal models with AP. There were 2 experiments (named "a" and "b," respectively) included in the meta-analysis, in the studies by Fu et al, 2018[43]; Kim et al, 2019[46]; Li et al, 2018[58]; and Li et al, 2018.[68,69] To distinguish them, the suffixes "a" and "b" were used in these studies. IV indicates inverse variance; Tau^2, τ^2 statistic; Chi^2, χ^2 statistic; *df*, degrees of freedom; I^2, I^2 heterogeneity statistic; *Z*, *Z* statistic.

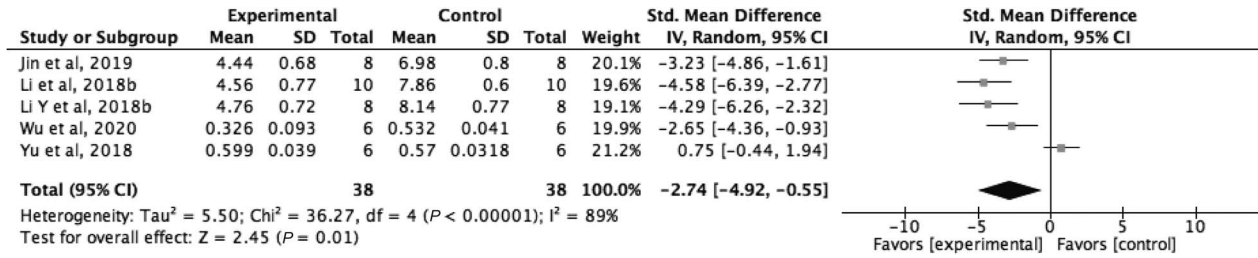

FIGURE 6. Forest plot of the effect of NLRP3 inflammasome inhibition on histopathological score of acute lung injury in animal models with AP. There were 2 experiments (named "a" and "b," respectively) included in the meta-analysis, in the studies by Li et al, 2018[58] and Li et al, 2018.[68,69] To distinguish them, the suffixes "a" and "b" were used in these studies. IV indicates inverse variance; Tau^2, τ^2 statistic; Chi^2, χ^2 statistic; *df*, degrees of freedom; I^2, I^2 heterogeneity statistic; *Z*, *Z* statistic.

differ significantly among the 3 animal models of AP (cerulein, cerulein + LPS, and sodium taurocholate–induced AP). Given the moderate or high heterogeneity of the included studies, the effects of NLRP3 inflammasome inhibition must be interpreted with some caution. The role of NLRP3 inflammasome in the pathogenesis and progression in AP and some promising therapeutic strategies targeting at NLRP3 inflammasome has been discussed comprehensively in previous study.[21] Hence, in our study, we focused on the protective effects of NLRP3 inflammasome inhibition on AP and tried to provide some intuitive, quantitative data on this.

There are multiple early-stage cellular events important to the pathogenesis of AP, including premature trypsinogen activation within acinar cells, pathological calcium signaling, mitochondrial dysfunction, endoplasmic reticulum stress, and overwhelmed oxidative stress, impaired autophagy, and so on.[70] In recent years, the important role of cell death, especially programmed cell death, has been found in a wide range of common clinical disorders, including stroke, myocardial infraction, sepsis, cancer, and pancreatitis.[71,72] Pancreatic acinar cells are the predominant cell type in the pancreas and the primary victims of injury induced by common acinar cell toxins, such as alcohol, nicotine, and bile acids.[73,74] Cell death by apoptosis does not lead to plasma membrane rupture and therefore does not induce an inflammatory response.[75,76] Apart from apoptosis, cell death can occur by unregulated necrosis or by other forms of programmed cell death including necroptosis[77] and pyroptosis.[78] In contrast to apoptosis, unregulated necrosis and other forms of programmed acinar cell death usually lead to the release of damage-associated molecular patterns that drive both local tissue inflammation and the activation of further acinar cell death. Following the initial events, cell death and inflammation can induce each other forming an autoamplification loop that causes exaggerated cell death and systemic inflammatory responses. This "necroinflammatory" environment promotes systemic inflammation, distant organ failure, and SAP.[11,79] In view of the crucial role of programmed cell death in AP, different modes of acinar cell death related to SAP have been extensively investigated, including autophagic cell death,[80] mitochondrial permeability transition-mediated regulated necrosis,[81] necroptosis,[77] and pyroptosis.[12] Pyroptosis is a caspase-dependent and highly immunogenic form of cell death, characterized by release of proinflammatory cytokines and cellular contents, and is mainly regulated by the canonical NLRP3 inflammasome pathway.[82,83] Exploring the underlying mechanisms further, the activation of NLRP3 inflammasome has been detected and the regulation of NLRP3 pathway has been evaluated in a significant number of studies.[12,50]

The strong inflammatory potential, as well as its critical role in driving cell death, makes NLRP3 inflammasome an attractive therapeutic target. To date, clinical treatments of NLRP3 inflammasome-involved diseases predominately target IL-1β with IL-1β antibodies or recombinant IL-1β receptor antagonists, such as canakinumab and anakinra.[84] However, it should be noted that IL-1β secretion is not the only product of NLRP3 inflammasome activation. Other inflammatory cytokines, such as IL-18, may also participate in the promotion of inflammation, and IL-1β can also be produced by other inflammatory pathways besides NLRP3 inflammasome, which means that complete inhibition of IL-1β might risk unintended immunosuppression.[19] Thus, pharmacological inhibitors specific to NLRP3 inflammasome may be a safer and more effective choice for NLRP3-related diseases. Fortunately, numerous promising inhibitors of NLRP3 inflammasome activation have been developed, including MCC950,[85] β-hydroxybutyrate,[86] type I interferon,[87] tranilast,[88] and CY-09,[89] to name a few. Among them, MCC950 is the most potent and specific NLRP3 inhibitor. A previous study showed that MCC950 specifically inhibits both canonical and noncanonical NLRP3 inflammasome activation and proinflammatory IL-1β secretion in human and mouse macrophages.[85] A recent study by Sendler et al[50] demonstrated that MCC950 administration could significantly reduce pancreatic injury, as well as systemic inflammatory response in an AP animal model. In addition, MCC950 exerts therapeutic efficacy against a variety of preclinical immunopathological models, such as Alzheimer disease, traumatic brain injury, atherosclerosis, myocardial infarction, diabetes, steatohepatitis, and colitis.[20,90] Despite the compelling evidence, a phase 2 clinical trial of MCC950 for rheumatoid arthritis was suspended due to hepatic toxicity.[19] (The specific cause is unclear, but the very high clinical dosage of 1200 mg/d and its metabolically reactive furan moiety are thought to underlie the observed toxicity.)[19] Given that there are other options for NLRP3 inhibition,[91] further translational studies are required using other NLRP3 inflammasome inhibitors against various NLRP3-related diseases, including AP.

This present study has several limitations. First, there are limited studies reported the effects of NLRP3 inflammasome inhibition on AP-associated distant organ failures (5 studies for acute lung injury and 3 studies for acute intestinal injury), which prevents drawing firm conclusions regarding the role of NLRP3 inflammasome in AP-associated organ failures. Second, the included studies varied in terms of AP animal models used, drugs targeted NLRP3 inflammasome, and the initiation of administration (predisease or after disease), which may contribute to the study heterogeneity. Finally, the risk of bias was unclear or high across the included studies, and study quality was judged to be low.

CONCLUSIONS

To conclude, NLRP3 inflammasome activation plays a critical role in the pathogenesis of AP, linking cell death and systemic inflammation. By meta-analysis of animal studies, NLRP3 inflammasome inhibition was shown to have protective effects on

AP. There is evidence at 3 levels: reduced local pancreatic injury (pancreatic histopathological scores, serum amylase, and lipase levels), reduced systemic inflammatory responses (circulating inflammatory cytokine levels), as well as organ failures (AP-associated acute lung injury and acute intestinal injury). These experimental studies indicate that inhibition of NLRP3 inflammasome is a promising therapeutic strategy for the treatment of AP. The role of pharmacological inhibition of NLRP3 activation and its clinical translation requires further studies.

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