Research article

Breaking the chain in organ failure: Role of umbilical cord and bone marrow derived mesenchymal stem cells in treatment of severe acute pancreatitis

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ARTICLE INFO

ABSTRACT

Background: Previous studies showed that MSCs could mitigate damage in the pancreas during acute pancreatitis (AP). However, acute mortality associated with AP was more often a result of persistent failure of remote organs, rather than local damage, especially in severe acute pancreatitis (SAP), and the effect of MSCs may vary depending on their origin.
Methods: An SAP model was induced in 8-week C57BL/6 J male mice by retrograde injection of 5 % sodium taurocholate solution through the bile duct. SAP mice were divided into the SAP group, UC-MSCs group, and BMSCs group, which were treated with saline, 1×10^6 UC-MSCs, and 1×10^6 BMSCs respectively, through the tail vein. After treatment, serum markers, inflammation, and morphology were assessed in the pancreas, kidneys, lungs, and hearts.
Results: MSCs infusion ameliorated the systemic inflammatory response in SAP mice. In the MSCs-treated SAP mice, local tissue injury and inflammation response in the pancreas were alleviated. But more importantly, the renal and lung injury were all significantly and drastically mitigated, and the levels of pro-inflammatory factors such as IL-6, MCP-1, IL-1β, and TNF-α in the kidney, lung and heart were sharply decreased. In terms of origin, UC-MSCs exhibited superior efficacy compared with BMSCs. Furthermore, compared to the normal control mice, UC-MSCs showed an earlier appearance, higher distribution densities, and longer duration of presence in the injured tissue.
Conclusions: This study provides compelling evidence supporting the therapeutic potential of MSCs in SAP treatment and particularly their ability to mitigate multi-organ failure. Our results also suggested that UC-MSCs may offer greater advantages over BMSCs in SAP therapy.

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1. Introduction

Acute pancreatitis (AP) is a common gastrointestinal disease with a global incidence of approximately 34 cases per 100,000 person-years [1]. The severity of AP can be mild, moderate, or severe, which depends on the extent of local injury in and around the pancreas, and more importantly systemic injury to remote organs. Severe acute pancreatitis (SAP) is defined as AP with persistent failure of an organ system due to systemic inflammation regardless of the severity of local injury [2]. Failure of remote organs (respiratory, cardiovascular, or renal) is considered the prime determinant of a poor outcome [3,4]. The overall mortality of AP is approximately 2 %, but it approaches 30 % among patients with SAP. Unfortunately, up till now, the management for organ failure (OF) in SAP remains largely supportive [5]. Effective treatment and intervention to retard or reverse OF progression is urgently needed.

Mesenchymal stem cells (MSCs) are adult, fibroblast-like multipotent cells characterized by the ability to differentiate into tissues of mesodermal origin [6]. In addition to their stem/progenitor properties, MSCs are more renowned for their broad immunoregulatory abilities. They are capable of influencing both adaptive and innate immune responses. Researchers have shown that MSCs interact with components of the innate immune system, displaying anti-inflammatory effects. MSCs proved effective in the treatment of various acute inflammatory diseases, including acute myocardial infarction, acute respiratory distress syndrome (ARDS), and acute myeloid leukemia [7–9].

Previous studies showed that MSCs could suppress inflammatory response and therefore mitigate local damage in the pancreas during AP. Jung KH et al. demonstrated that MSCs reduced follicular cell degeneration, pancreatic edema, and inflammatory cell infiltration in AP rats with a decrease in the expression of inflammatory mediators and cytokines [10]. Similarly, Huang Q et al. confirmed that pancreatic tissue damage and systemic inflammation were significantly alleviated in AP rats after infusion of MSCs [11]. However, as mentioned above, the systemic severity can precede local severity and the extent of pancreatic necrosis may not correlate with systemic injury in SAP [12]. OF, rather than local injury, governs the outcome and mortality in patients with AP [5]. It remains elusive whether MSCs infusion can ameliorate OF in SAP.

Nonetheless, inflammation is the key pathophysiological response both at the local and systemic levels in SAP, and the systemic injury is a result of dysregulated and out-of-proportion systemic inflammation in response to the local injury. In patients with AP, systemic inflammation presents initially as systemic inflammatory response syndrome (SIRS). Patients with persistent SIRS are prone to develop systemic organ dysfunction and later OF. We therefore hypothesize that MSCs infusion could inhibit systemic inflammation and therefore prevent, retard, or reverse the development of renal, respiratory, and cardiovascular failure.

Additionally, studies have shown that MSCs from different sources demonstrate distinct biological characteristics [13]. Bone marrow-derived MSCs (BMSCs) and umbilical cord-derived MSCs (UC-MSCs) exhibit differential gene expression profiles and paracrine factor secretion. Pathway analysis indicated that UC-MSCs have a higher immunomodulatory potential, while BMSCs showed greater potential in supporting regenerative processes [14]. MSCs of different origins acted differently on the treatment of the same disease [15,16]. We thereby further hypothesize that UC-MSCs could offer greater advantages over BMSCs in the treatment of SAP.

2. Subjects and methods

2.1. Isolation and identification of UC-MSCs and BMSCs

Fresh umbilical cords were obtained from healthy pregnant women who had undergone cesarean section at the Chinese PLA General Hospital after obtaining informed consent. The study was approved by the Ethics Committee of the Chinese PLA General Hospital. The Wharton's jelly, which is the remaining part after removing the outer membrane, the umbilical artery, and the umbilical vein, was cut into pieces and placed in a culture dish. 10 ml of minimum essential medium (MEM; Gibco, USA) containing 10 % UltraGRO™-Advanced (Helios, Bioscience) was added, and the dish was incubated at 37 °C with 5 % CO_2. After one week, non-adherent tissues and cells were discarded, and the remaining adherent cells were washed twice with saline. Then fresh medium was added, and the cells were passaged according to their growth. UC-MSCs were characterized for their phenotype and ability to differentiate into adipocytes and osteoblasts, as our team previously reported [17]. Mouse C57BL/6 J BMSCs were purchased from Cyagen Biosciences (MUBMX-01001, 220608A51, USA). CM-Dil (chloromethyl-indocarbocyanine, Life Technologies, Eugene, Oregon, USA) labeled MSCs were administered to SAP mice to investigate MSCs homing in organs.

2.2. Surgical procedure of SAP model

Male C57BL/6 J mice, 8 weeks old and weighing 22–25 g, were used for the study. On the day of surgery, a fresh solution of 5 % sodium taurocholate (S0900000 Sigma-Aldrich, USA) in saline, with added methylene blue staining solution at a concentration of 0.1 % (Sigma-Aldrich, USA), was prepared. The mice were anesthetized with 1.5 % isoflurane (RWD, Shenzhen, China). After disinfection, a midline incision was made to access the abdominal cavity. The bile duct was occluded near the hepatic hilum using a vascular clip. A 30G needle connected to a microinjection pump (Mindray, China) was inserted into the bile duct through the intestinal wall, and the prepared solution was pumped at a flow rate of 5 μl/min for 10 min. Successful cannulation and infusion were visualized by the appearance of scattered methylene blue staining in the pancreas. The abdominal cavity was then closed with suture (Fig. 1A). The entire procedure was completed within 30 min [18].
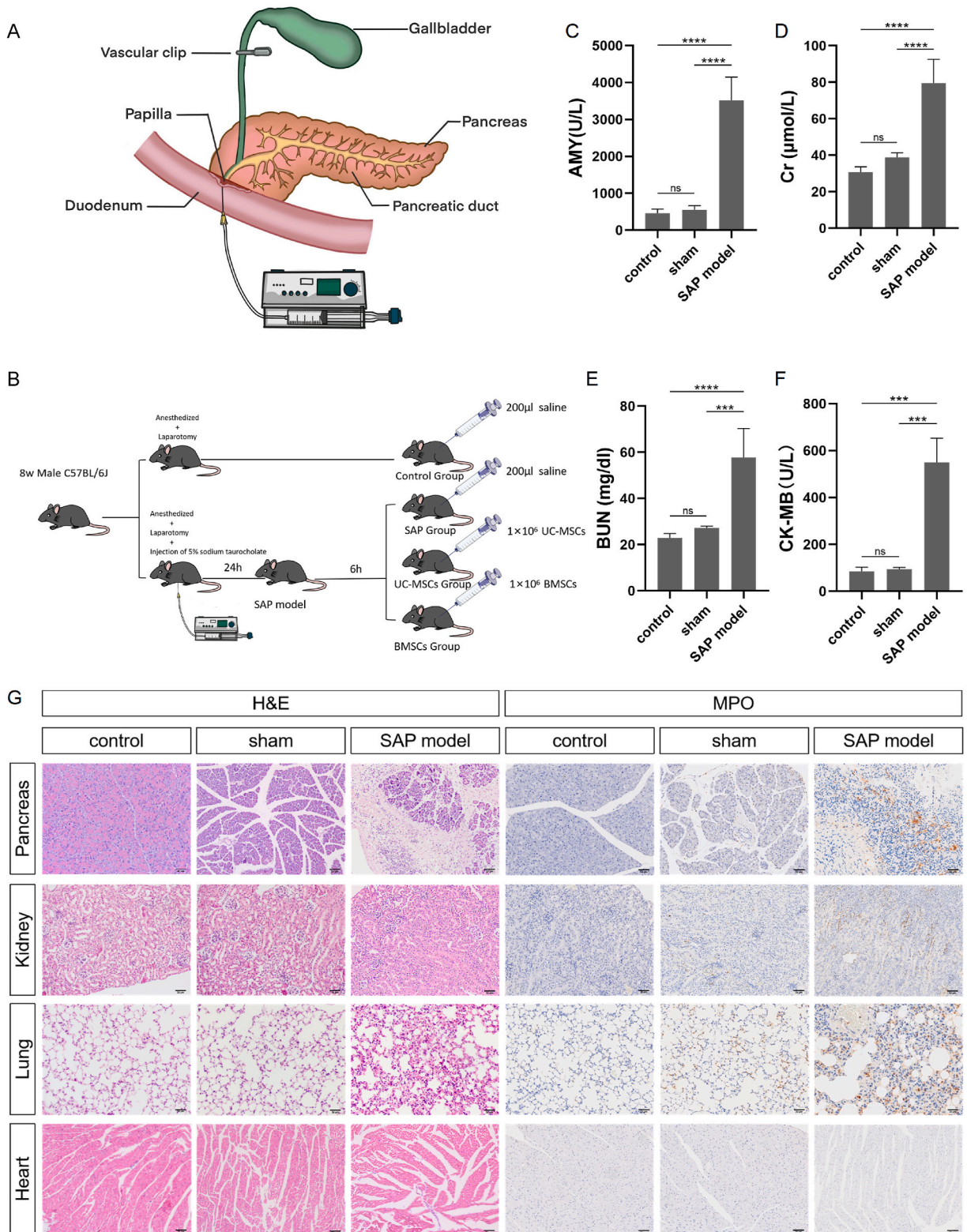
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Fig. 1. Surgical procedure of SAP model (A) and MSCs treatment (B). Establishment of SAP mouse model. Changes in serum levels of AMY, Cr, BUN, and CK-MB (C–F). Representative tissue sections by H&E and MPO staining; bars = 50 μm (G). The mice in the control group refer to completely normal mice without any treatment, the mice in the sham group underwent anesthesia, laparotomy, and bile duct puncture without fluid injection, and the mice in the SAP model group underwent bile duct injection of 5 % sodium taurocholate solution. The mice in the sham group and the SAP model group were euthanized 24h after the laparotomy. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

2.3. 2.3 SAP animal models

To confirm the establishment of the SAP model by the procedure, 6 mice underwent bile duct injection of 5 % sodium taurocholate solution (SAP model) and were euthanized 24h after the laparotomy, 6 mice underwent anesthesia, laparotomy, and bile duct puncture without fluid injection (sham) and were euthanized 24h after the laparotomy [18,19]. Mice with anesthesia and laparotomy were used as normal controls. Local damage in the pancreas and systemic damage to the kidneys, lungs, and cardiac tissue were identified by serum and histopathological analysis.

2.4. MSCs treatment

The experimental animals were randomly assigned to 4 treatment groups, all animals underwent anesthesia, laparotomy with or without the procedure (bile duct injection of 5 % sodium taurocholate solution), and received tail vein infusion of saline, UC-MSCs, and BMSCs 30h after the laparotomy, euthanized 48h after infusion. In detail: 1. Control group (n = 6): Mice underwent anesthesia, and laparotomy without the procedure, received 200 μl saline infusion, and were euthanized 48h after infusion. 2. SAP-Saline treatment group (SAP group, n = 6): Mice underwent anesthesia and the procedure, received 200 μl saline infusion and were euthanized 48h after infusion; 3. SAP-UC-MSCs treatment group (UC-MSCs group, n = 6): Mice underwent anesthesia and the procedure, received a tail vein injection of 200 μl UC-MSCs (approximately 1×10^6 cells) and were euthanized 48h after infusion; 4. BMSCs treatment group (BMSCs group n = 6): Mice underwent anesthesia and the procedure, received a tail vein injection of 200 μl BMSCs (approximately 1×10^6 cells) and were euthanized 48h after infusion (Fig. 1B).

2.5. Serum tests

Blood samples were collected from the ophthalmic artery. After resting at room temperature for 2 h, the samples were centrifuged at 4000 g for 15 min at 4 °C to collect the serum. The serum levels of amylase (AMY), creatinine (Cr), blood urea nitrogen (BUN), and creatine kinase MB isoenzyme (CK-MB), were measured according to the protocol by corresponding reagent kits purchased from the Servicebio corporation.

2.6. ELISA

The levels of cytokines (IL-6, MCP-1, IL-1β, TNF-α, and IL-10) in the serum were measured using ELISA kits (Neobioscience Technology Co. Ltd, Beijing, China). Mice were perfused with BPS. Pancreatic, kidney, lung, and heart tissues were collected and homogenized by grinding with 9 times the volume of homogenization medium. The resulting solution was then centrifuged at 3000-4000r for 10 min. The supernatant was used to prepare a 10 % tissue homogenate. The levels of cytokines (IL-6, MCP-1, IL-1β, TNF-α, and IL-10) in the tissue homogenates were determined using ELISA kits (Neobioscience Technology Co. Ltd, Beijing, China).

2.7. Quantitative real-time reverse transcriptase polymerase chain reaction

The total RNA was extracted from tissues using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, USA). The RNA was then

Table 1
qRT-PCR Primer Sequences.

Mouse			
Genes	Direction	Primer Sequence (5'-3')	Length(bp)
GAPDH	F	CCTCGTCCCGTAGACAAAATG	133
	R	TGAGGTCAATGAAGGGGTCGT	
TNFα	F	CCCTCACACTCACAAACCACC	93
	R	CTTTGAGATCCATGCCGTTG	
MCP-1	F	AGAAGCTGTAGTTTTTGTCACCAAG	192
	R	GCTTCAGATTTACGGGTCAACT	
IL-6	F	CCCCAATTTCCAATGCTCTCC	141
	R	CGCACTAGGTTTGCCGAGTA	
IL-1β	F	AGGCTCCGAGATGAACAACAAA	206
	R	GTGCCGTCTTTCATTACACAGGA	
IL10	F	CACTGCTATGCTGCCTGCTCT	191
	R	GTCGGTTAGCAGTATGTTGTCCAG	

reverse-transcribed into single-stranded cDNA using a reverse transcription kit (Thermo Scientific, Waltham, MA), following the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was performed on an ABI Prism thermal cycler (Applied Biosystems, CA, USA) using SYBR® Green PCR Master Mix (Applied Biosystems). The thermal cycling program consisted of 40 cycles of incubation at 50 °C for 2 min, followed by 94 °C for 5 min, 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s. Melting curve analysis was conducted to ensure primer specificity. Gene expression levels were normalized to the GAPDH level. The primers used are listed in Table 1.

2.8. Hematoxylin-eosin and myeloperoxidase staining

Pancreatic, kidney, lung, and heart tissues were fixed in 10 % formalin and embedded in paraffin. Sections measuring 4 mm in thickness were prepared and stained with Hematoxylin-eosin (H&E) as well as anti-myeloperoxidase (MPO) (1:2000, mouse, Abcam). The morphological structure of each tissue was observed using a light microscope (Olympus, Japan), and histological analysis was performed. The severity score for pancreatic injuries followed Schmidt's histopathologic scoring criteria [20], briefly based on edema, acinar necrosis, hemorrhage and fat necrosis, inflammation, and perivascular infiltrate. Renal injury was assessed based on the percentage showing cellular necrosis, loss of brush border, cast formation, and tubular dilatation: $0 = $ none, $1 \leq 10$ %, $2 = 11$–25 %, $3 = 26$–45 %, $4 = 46$–75 %, $5 \geq 76$ % [21]. Lung injuries were scored on a 0–2 scale [22], briefly based on neutrophils infiltration, hyaline membranes, proteinaceous debris, and alveolar septal thickening. Cardiac injuries were assessed based on a 0–4 scoring method [23], briefly including interstitial edema, hemorrhage, and neutrophil infiltration.

2.9. Immunofluorescence staining

CM-dil labeled UC-MSCs (1×10^6) were injected into mice with or without SAP. After euthanized by intraperitoneal injection of 1 % sodium pentobarbital (50 mg/kg) at 6h, 12h, 24h, 3d, 5d, and 7d post-infusion, respectively, mice were perfused with 10 ml of PBS through the left ventricle, followed by 15 ml of 4 % paraformaldehyde. After perfusion, the pancreas, kidneys, lungs, and hearts were isolated and incubated in 30 % sucrose/PB overnight. The tissues were then embedded with optimal cutting temperature compound (OCT, Sakura, Finetek, USA) to make 6 μm frozen sections. Cell nuclei were stained with DAPI, and immunofluorescence-stained sections were examined and photographed using a laser scanning confocal microscope (Leica, Wetzlar, Germany).
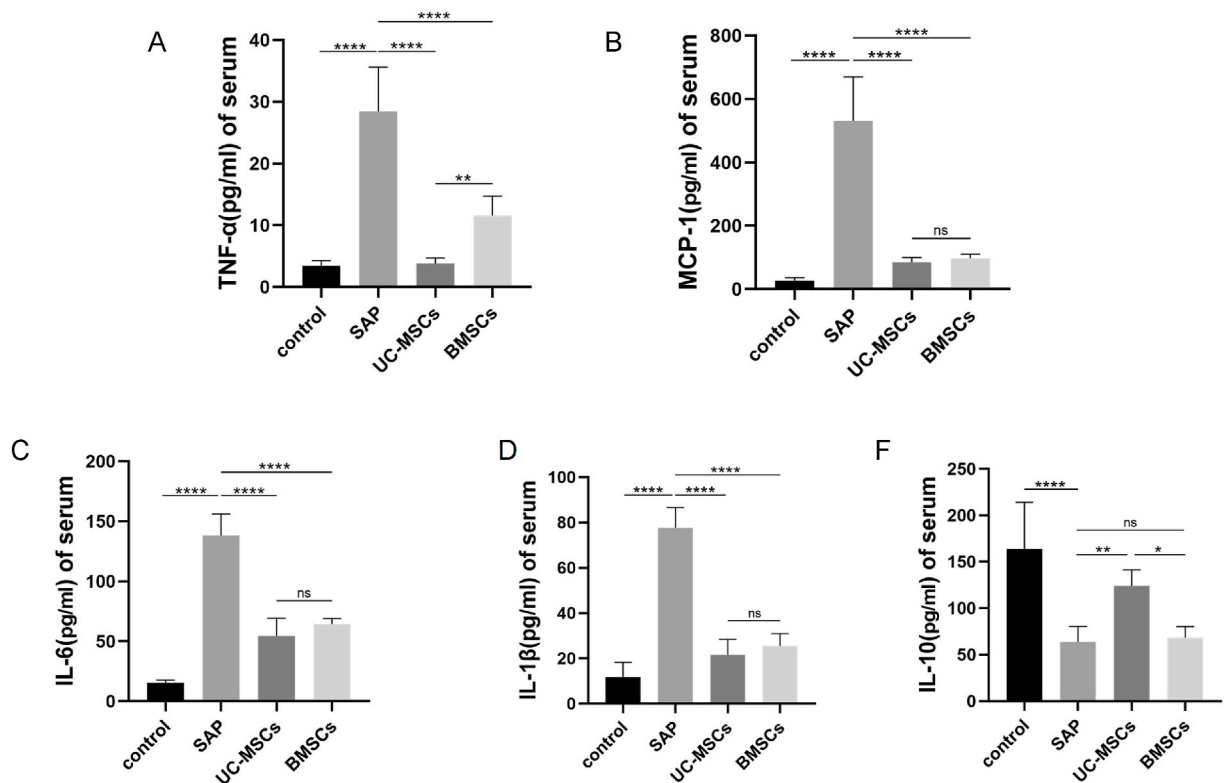

Fig. 2. UC-MSCs infusion is more able to alleviate systemic inflammatory response in SAP mice compared with BMSCs infusion. Changes in serum levels of TNF-α, MCP-1, IL-6, IL-1β, and IL-10 (A–F). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
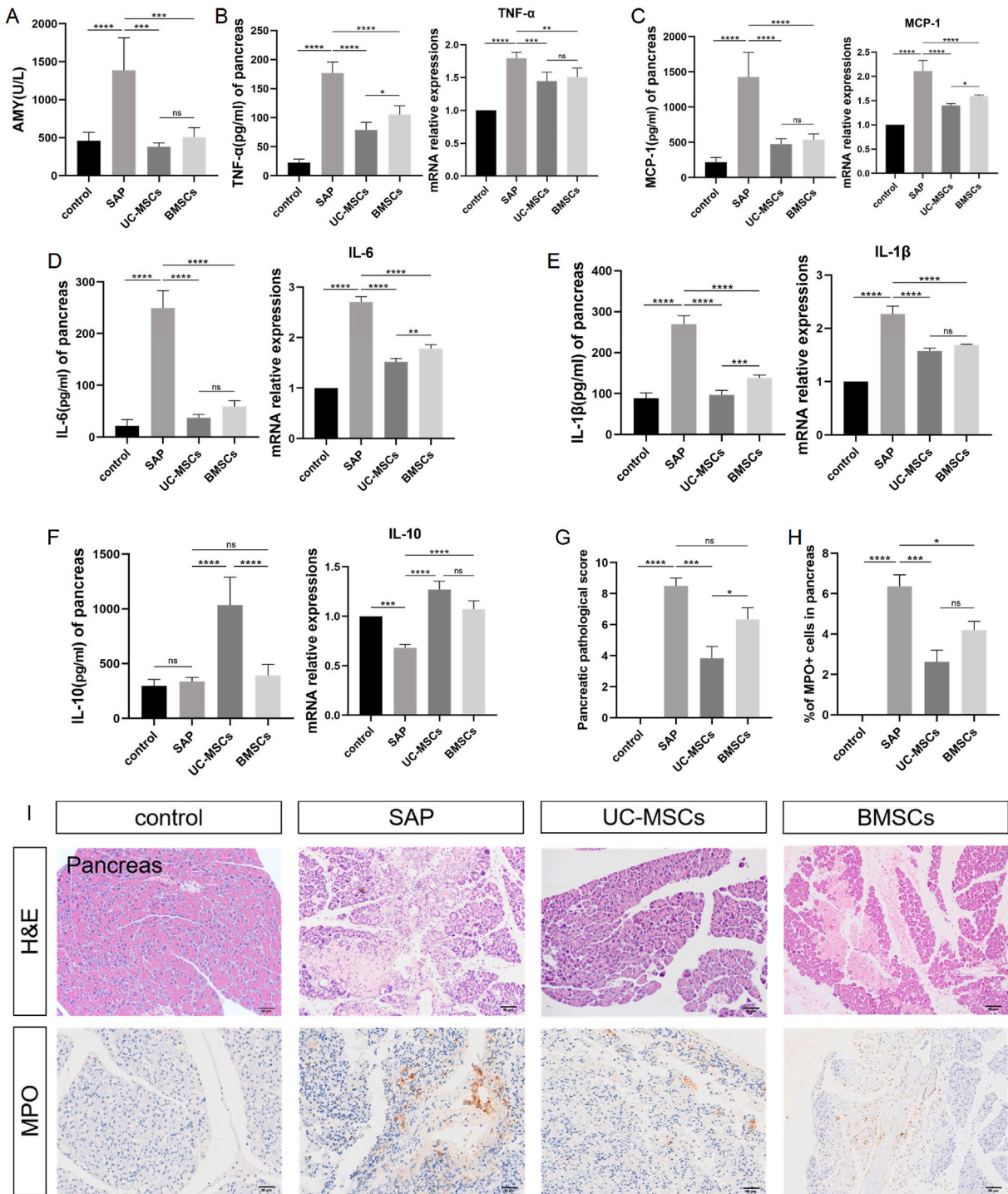
Fig. 3. UC-MSCs infusion is more effective in relieving pancreatic injury in SAP mice compared with BMSCs Infusion. Changes in serum levels of AMY (A). Changes in TNF-α, MCP-1, IL-6, IL-1β and IL-10 levels in pancreatic tissues and quantitative reverse transcription-polymerase chain reaction analysis of gene expression in the control group, SAP group, UC-MSCs group and BMSCs group (B–F). Representative pancreatic tissue sections by H&E and MPO staining; bars = 50 μm (I). Pancreatic histopathological scores (G) and quantitative analysis of MPO + cells (H). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
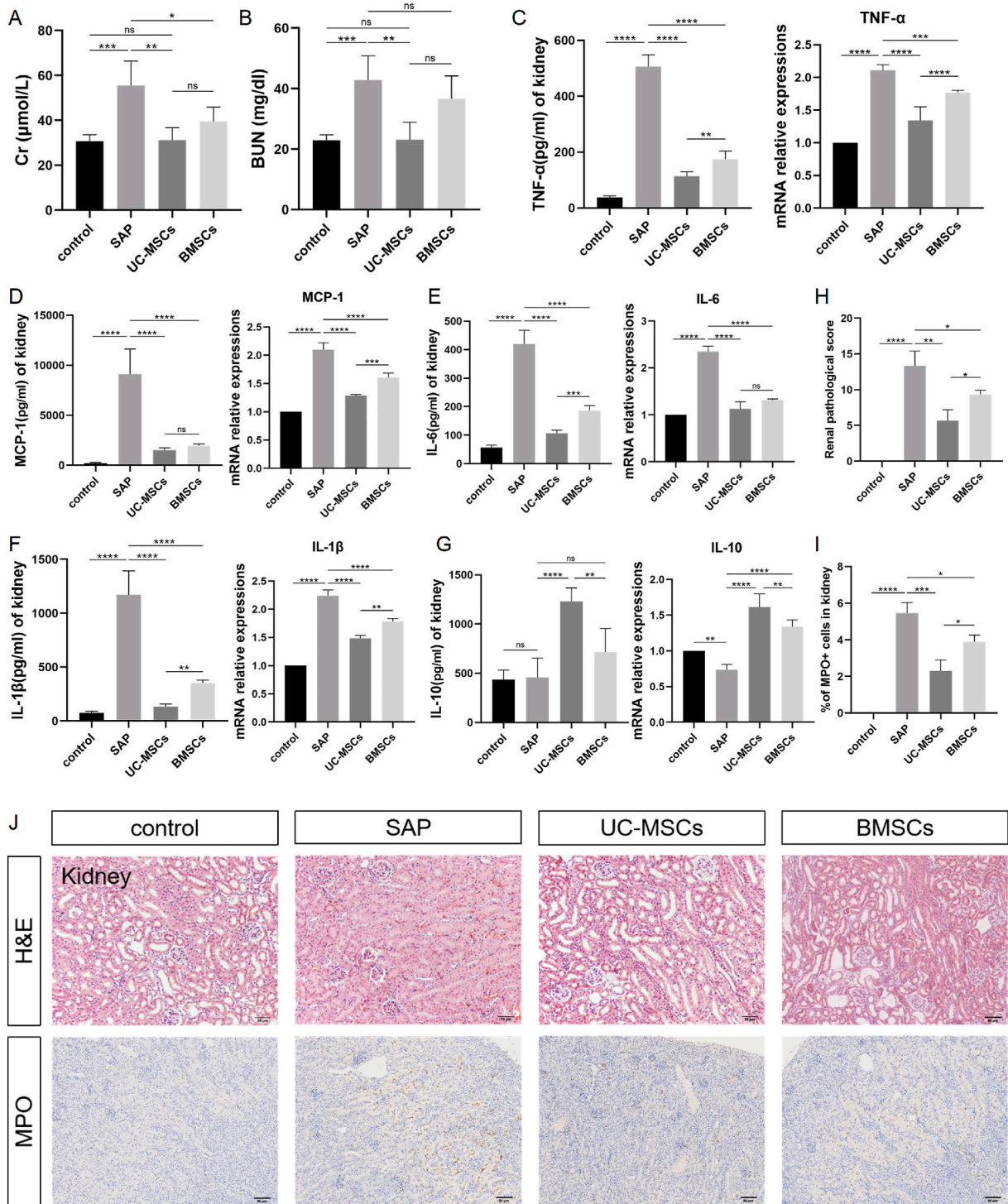
Fig. 4. UC-MSCs infusion is more valid in mitigating renal injury in SAP mice compared with BMSCs infusion. Changes in serum Cr and BUN levels (A–B). Changes in TNF-α, MCP-1, IL-6, IL-1β and IL-10 levels in renal tissues and quantitative reverse transcription-polymerase chain reaction analysis of gene expression in the control group, SAP group, UC-MSCs group and BMSCs group (C–G). Representative kidney tissue sections by H&E and MPO staining; bars = 50 μm (J). Renal histopathological scores (H) and quantitative analysis of MPO + cells (I). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

2.10. Statistical analysis

SPSS 23.0 software was used to analyze the data. Quantitative data were expressed as mean ± standard deviation (SD). Normally distributed data were compared between groups using an unpaired *t*-test and non-normally distributed data using the Mann-Whitney *U* test. One-way ANOVA with a post hoc Fisher's least-significant-difference (LSD) test was conducted for comparison between groups. *P* < 0.05 was considered statistically significant.

3. Results

3.1. Establishment of SAP mouse model

After bile duct injection of 5 % sodium taurocholate solution, the SAP model was allowed to progress for 24 h. At 24h post-injection, there was a drastic increase in serum levels of amylase, creatinine, blood urea nitrogen, and CK-MB in the SAP group but not in the other groups (*P < 0.05*, Fig. 1C–F). The histopathological findings showed evident tissue damage in the pancreases, kidneys, and lungs of the model mice, with MPO staining indicating infiltration of neutrophils in the injured tissues, compared with control and sham group (Fig. 1G). Of note, no discernible histopathological damage or neutrophil infiltration was observed in the cardiac tissue of SAP mice (Fig. 1G). No significant difference was detected between the control and sham group. These findings indicated the successful establishment of the SAP mouse model 24 h after bile duct injection of 5 % sodium taurocholate solution.

3.2. MSCs infusion significantly alleviate systemic inflammatory response in SAP mice, with UC-MSCs showing higher efficiency than BMSCs

In the SAP group, ELISA showed that serum levels of pro-inflammatory cytokines (TNF-α, MCP-1, IL-6, IL-1β) increased, while the anti-inflammatory factor (IL-10) was decreased. After MSCs treatment, the upregulation of TNF-α, MCP-1, IL-6, and IL-1β were all significantly and dramatically mitigated (*P < 0.05*, Fig. 2A–D), while the depressed expression of IL-10 was significantly alleviated (*P < 0.05*, Fig. 2F). These results demonstrated that MSCs infusion significantly alleviate systemic inflammatory response in SAP mice. Notably, UC-MSCs treatment resulted in a stronger effect in repressing serum TNF-α and elevating IL-10 levels, while BMSCs infusion did not significantly reverse the decrease in IL-10 serum level (*P < 0.01*, Fig. 2A–F). These results indicated that UC-MSCs could be more effective in ameliorating systematic inflammation response in SAP than BMSCs.

3.3. MSCs infusion effectively relieved pancreatic injury in SAP mice, with UC-MSCs showing higher efficiency than BMSCs

To investigate the effect of MSCs in relieving pancreatic injury, we evaluated serum amylase, tissue TNF-α, MCP-1, IL-6, IL-1β, and IL-10 levels and histology of the pancreas. The SAP group showed evident serum amylase increase, which was suppressed after MSCs treatment (*P < 0.001*, Fig. 3A). On the transcriptional level, qRT-PCR analysis showed that MSCs treatment resulted in significantly lower TNF-α, MCP-1, IL-6, IL-1β levels and higher IL-10 levels in the pancreatic tissue (*P < 0.05*, Fig. 3B–F). Similarly, on protein level, ELISA showed MSCs treatment resulted in significantly lower TNF-α, MCP-1, IL-6, and IL-1β levels in the pancreas tissue (*P < 0.0001*, Fig. 3B–F). Specially, only UC-MSCs infusion resulted in significantly higher IL-10 levels (*P < 0.0001*, Fig. 3F), but not BMSCs. Histology showed significantly lower pathological score and less pancreatic necrosis after treatment with UC-MSCs but not BMSCs (*P < 0.05*, Fig. 3G–I), while MPO staining indicated both MSCs were able to mitigate neutrophil infiltration in the pancreas (Fig. 3H and I). In addition, compared to BMSCs, UC-MSCs treatment also resulted in significantly lower transcriptional levels of MCP-1 and IL-6, lower protein levels of TNF-α, and IL-1β, and higher transcriptional and protein levels of IL-10. (*P < 0.05*, Fig. 3B–F). Taken together, these results suggested that MSCs infusion effectively relieved pancreatic injury in SAP mice, with UC-MSCs showing greater therapeutic potential than BMSCs.

3.4. MSCs infusion mitigated renal injury in SAP mice, with UC-MSCs showing higher efficiency than BMSCs

The kidney is one of the most frequently affected organs in SAP [24] and prominent acute kidney injury is also observed in our SAP model. Serum levels of creatinine and blood urea nitrogen increased rapidly and drastically in the SAP group but were significantly mitigated after MSCs treatment, and they almost drop to normal levels, especially after UC-MSCs treatment (*P < 0.05*, Fig. 4A–B). On the transcriptional level, qRT-PCR analysis showed that MSCs treatment resulted in significantly lower TNF-α, MCP-1, IL-6, and IL-1β levels and higher IL-10 levels in the renal tissue (*P < 0.05*, Fig. 4C–G). Similarly, on protein level, ELISA showed MSCs treatment resulted in significantly lower TNF-α, MCP-1, IL-6, IL-1β levels and higher IL-10 levels in the renal tissue (*P < 0.05*, Fig. 4C–G). Histology showed significantly lower renal pathological scores after treatment with MSCs (*P < 0.05*, Fig. 4H), while MPO staining indicated MSCs were able to inhibit neutrophil infiltration in the kidney (*P < 0.05*, Fig. 4I–J). In addition, compared to BMSCs, UC-MSCs treatment resulted in lower transcriptional levels of TNF-α, MCP-1, and IL-1β, lower protein levels of TNF-α, IL-6, IL-1β, and higher transcriptional and protein levels of IL-10 (*P < 0.05*, Fig. 4C–G). In terms of histology and neutrophil infiltration, the improvement induced by UC-MSCs is more evident than BMSCs (*P < 0.05*, Fig. 4H–I). Taken together, these results suggested that MSCs infusion effectively mitigated renal injury in SAP mice, with UC-MSCs showing superior therapeutic effects than BMSCs.
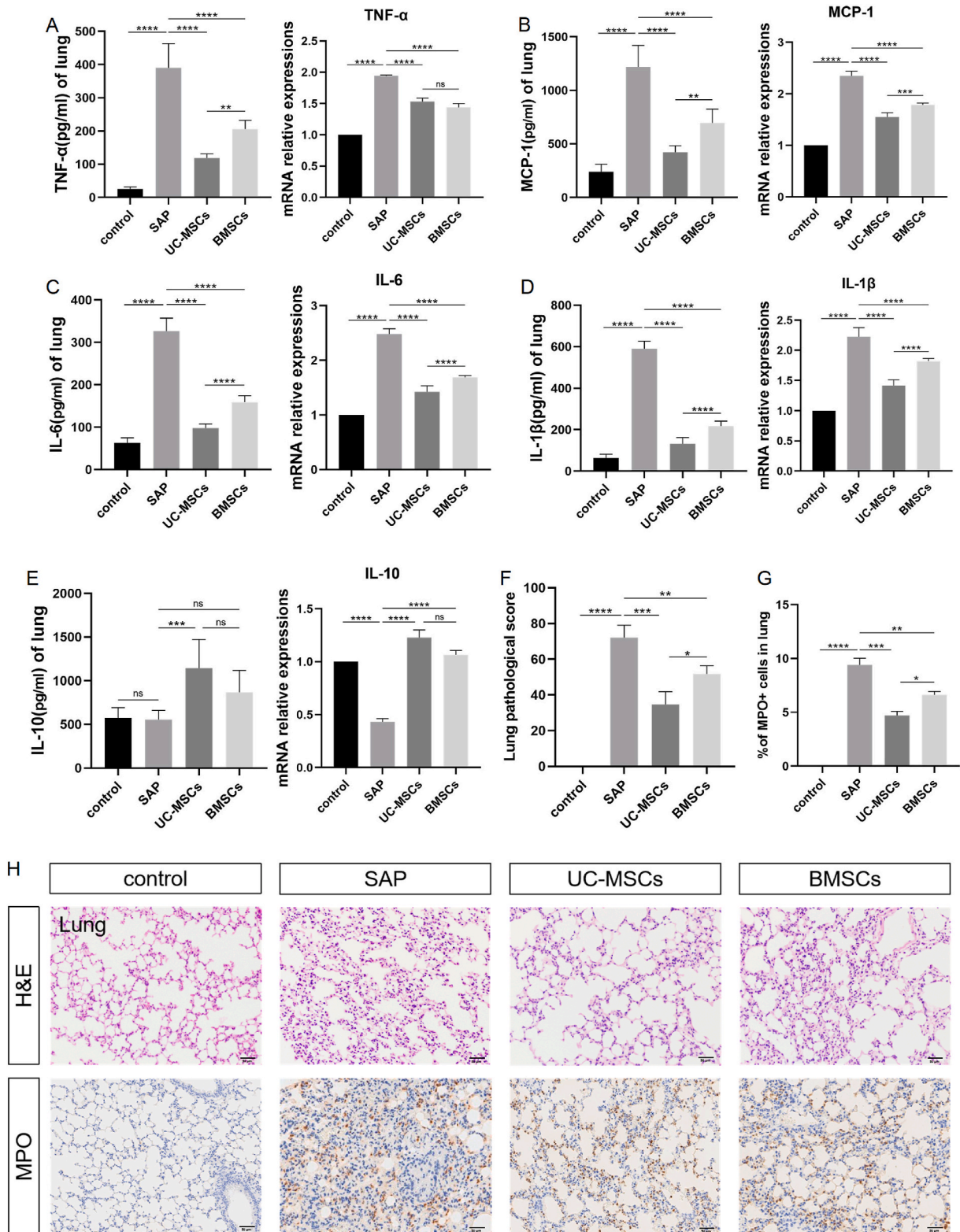
Fig. 5. UC-MSCs infusion showed better therapeutic effects in lung injury in SAP mice compared with BMSCs infusion. Changes in TNF-α, MCP-1, IL-6, IL-1β and IL-10 levels in lung tissues and quantitative reverse transcription-polymerase chain reaction analysis of gene expression in the control group, SAP group, UC-MSCs group and BMSCs group (A–E). Representative lung tissue sections by H&E and MPO staining; bars = 50 μm (H). Lung histopathological scores (F) and quantitative analysis of MPO + cells (G). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
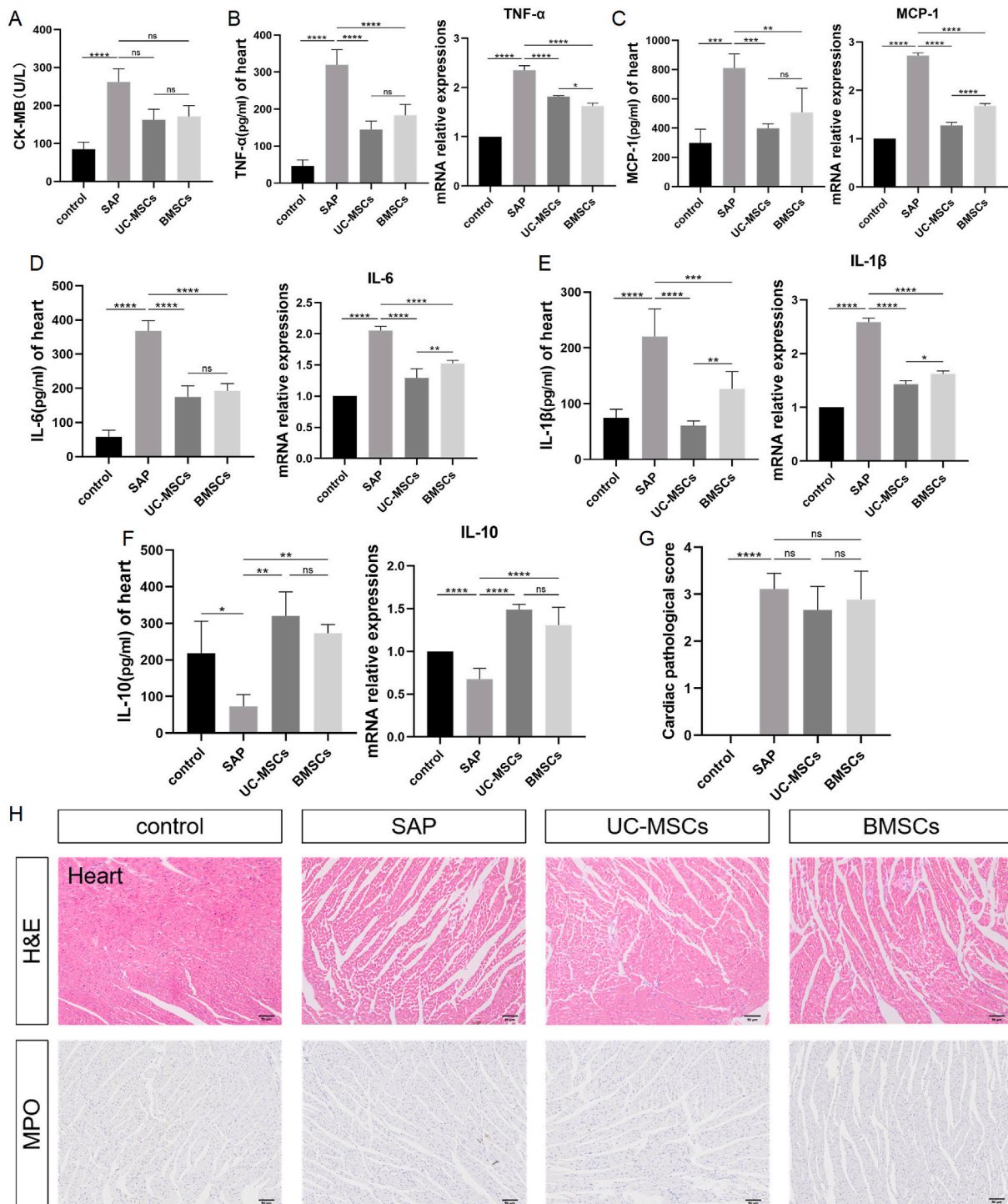
Fig. 6. UC-MSCs relieving cardiac injury better than BMCs in SAP mice. Changes in serum CK-MB levels (A). Changes in TNF-α, MCP-1, IL-6, IL-1β and IL-10 levels in cardiac tissues and quantitative reverse transcription-polymerase chain reaction analysis of gene expression in the control group, SAP group, UC-MSCs group and BMSCs group (B–F). Representative heart tissue sections by H&E and MPO staining; bars = 50 μm (H). Cardiac histopathological scores (G). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.*

3.5. MSCs infusion led to alleviated lung injury in SAP mice, with UC-MSCs showing better therapeutic effects than BMSCs

The lung is another commonly affected organ in SAP. Acute lung injury and acute respiratory distress syndrome are associated with significantly higher mortality in SAP [25]. Evident lung injury was rapidly induced in our SAP model. Interstitial thickening, alveolar destruction, and inflammatory cell infiltration were observed, which was significantly alleviated after MSCs treatment. On transcriptional level, qRT-PCR analysis showed that MSCs treatment resulted in significantly lower TNF-α, MCP-1, IL-6, IL-1β levels and higher IL-10 levels in the lung tissue ($P < 0.05$, Fig. 5A–E). Similarly, on protein level, ELISA showed MSCs treatment resulted in significantly lower TNF-α, MCP-1, IL-6, IL-1β levels in the lung tissue ($P < 0.05$, Fig. 5A–D), while UC-MSCs resulted in significantly higher IL-10 levels ($P < 0.05$, Fig. 5E), but not BMSCs. Histology showed significantly lower lung pathological scores after treatment with MSCs ($P < 0.05$, Fig. 5F), at the same time MPO staining indicated MSCs were able to inhibit neutrophil infiltration in the lung
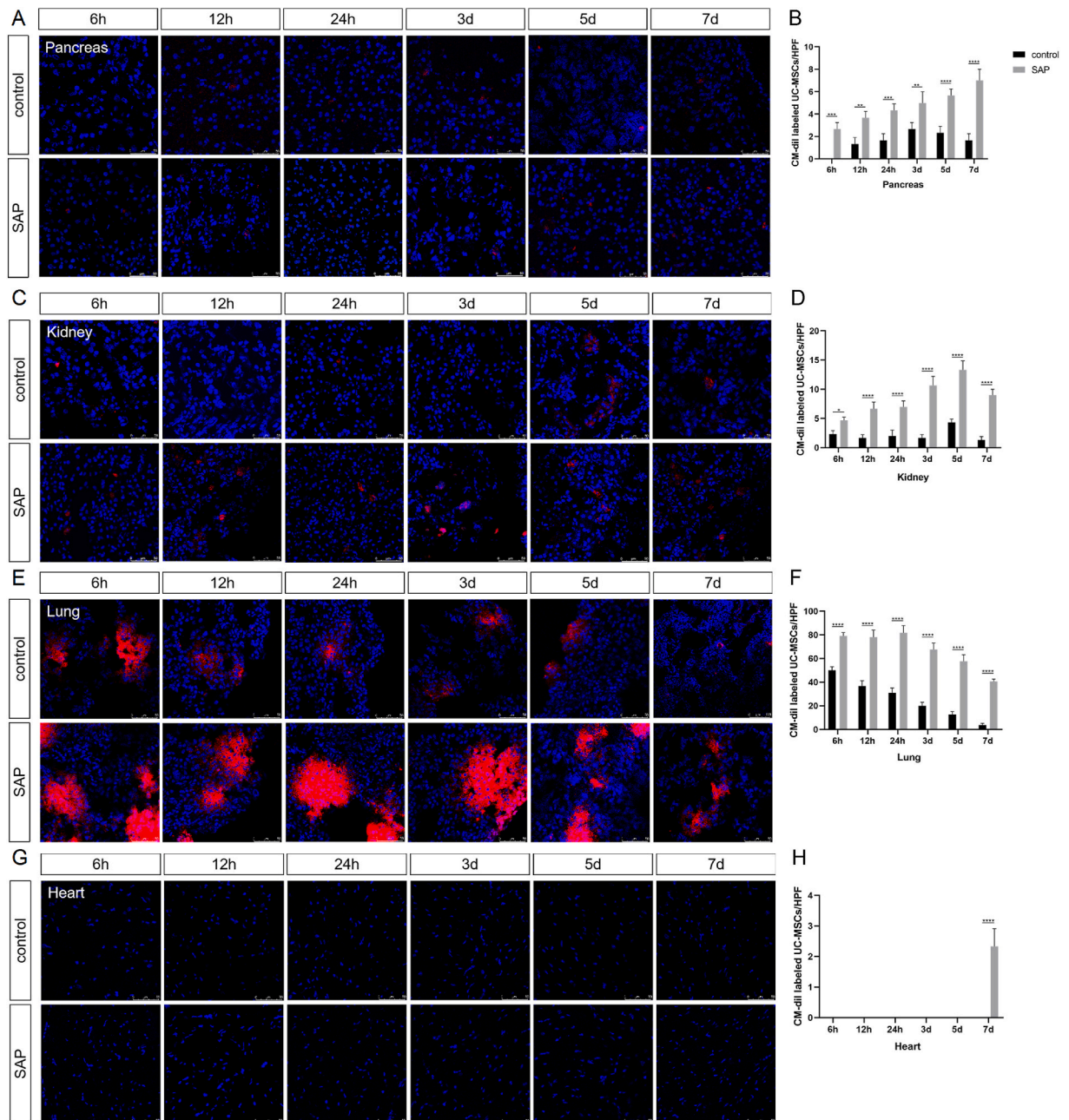

Fig. 7. Distribution of UC-MSCs and its assessment in the pancreas, kidney, lung and heart at 6h, 12h, 24h, 3d, 5d, and 7d post-infusion, respectively; bars = 50 μm, (A–D). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

($P < 0.05$, Fig. 5G–H). In addition, compared to BMSCs, UC-MSCs treatment resulted in lower transcriptional levels of MCP-1, IL-6, and IL-1β, lower protein levels of TNF-α, IL-6, IL-1β, and higher transcriptional and protein levels of IL-10 ($P < 0.05$, Fig. 5A–E). In terms of histology, the improvement induced by UC-MSCs is more evident than BMSCs ($P < 0.05$, Fig. 5F–H). In addition, UC-MSCs did lead to less prominent neutrophil infiltration than BMSCs ($P < 0.05$, Fig. 5G–H). Taken together, these results suggested that MSCs infusion effectively mitigated lung injury in SAP mice. Similar to the effect on pancreatic and renal injury, UC-MSCs showed better performance in improving lung injury than BMSCs.

3.6. UC-MSCs relieved cardiac injury better than BMCs in SAP mice

It has been reported that SAP or sepsis would lead to myocardial dysfunction or circulatory failure with transient elevation of serum markers [23,26]. Similarly, we detected a serum CK-MB increase in the SAP group, which decreased spontaneously when left untreated. The serum CK-MB level further declined after MSCs treatment, although statistically insignificant (Fig. 6A). In terms of histology, only myocardial interstitial edema was observed in the SAP group, and there was no treatment effect whatsoever (Fig. 6G). Nonetheless, SAP did lead to a marked change in levels of inflammatory factors, which could be responsible for myocardial dysfunction or circulatory failure. MSCs treatment resulted in significantly lower TNF-α, MCP-1, IL-6, IL-1β and higher IL-10 in the myocardial tissue on both transcriptional and protein levels ($P < 0.05$, Fig. 6B–F). Notably, UC-MSCs showed superior performance on altering TNF-α, MCP-1, IL-6, IL-1β, and IL-10 on transcription level, and IL-1β on protein level ($P < 0.05$, Fig. 6B–F). Taken together, these results suggested that although the cardiac effect was mild and transient in our model, MSCs infusion effectively suppressed inflammatory response in the cardiac tissue. UC-MSCs appeared to have a stronger effect on cardiac tissue than BMSCs.

3.7. Homing of UC-MSCs in the pancreas and related organs during SAP

We infused CM-Dil labeled UC-MSCs in normal and SAP mice to investigate the homing of UC-MSCs. We found significantly enhanced engraftment of UC-MSCs to the pancreas, the kidney, and the lung in the SAP group compared with control mice. In the control group, UC-MSCs hardly homed to the pancreas and kidney. While in the SAP group, UC-MSCs were detectable in the pancreas and kidney 6h after infusion with a gradual increase afterwards ($P < 0.05$, Fig. 7A–D). Homing to the lung was remarkable in both control and SAP group. Despite a gradual decrease since 24 h, the number of UC-MSCs in the lung remained impressive 7 days after infusion in SAP group, constantly higher than control group ($P < 0.05$, Fig. 7E–F). UC-MSCs homing to the heart was hardly detectable in control and SAP group throughout the process (Fig. 7G–H).

4. Discussion

It is well known that failure of remote organs largely dictates the prognosis of SAP [1,3,27]. Yet up till now, there is no specific therapy available either to treat or prevent the development of, and most of the specific treatment aimed at putative critical pathways failed to provide any therapeutic benefit. OF remains the proverbial Achilles heel of managing patients with SAP [5,25]. The present study demonstrates that MSCs treatment profoundly inhibited inflammatory response in the pancreas, the kidney, the lung, and the heart, and mitigated injuries to these organs, which could reduce the incidence of or even avoid OF. Furthermore, we proved that UC-MSCs could offer greater advantages over BMSCs in the treatment of SAP.

Although there have been attempts to treat AP with MSCs, most of the researchers focused on the local effect in alleviating pancreatic damage [10,12,28,29], which was not even related to the severity of SAP [25,30], while damages to remote organs were rarely investigated. Nonetheless, reducing pancreatic necrosis may potentially diminish the necessity of surgical intervention [31,32], or offer improved quality of life [33]. Consistent with previous reports [11,34,35], we found that MSCs infusion led to less prominent inflammation in the pancreas, along with reduced neutrophil infiltration. In addition, we also found that UC-MSCs infusion effectively reduced pancreatic pathological damage and necrosis. These indicated that treatment with MSCs at an early stage, especially UC-MSCs, may result in less exocrine and endocrine insufficiency in the later phase of pancreatitis by suppressing the local reaction and reducing pancreatic necrosis.

The systematic response and remote OF induced by SAP were actually the results of SIRS, though the precise mechanism remains unclear. As expected, in our study, MSCs infusion exhibited potent immunoregulatory abilities, inhibiting pro-inflammatory cytokines (TNF-α, MCP-1, IL-6, IL-1β) and upregulating anti-inflammatory factor (IL-10) in SAP mice, which would therefore limit further damages to other organ systems [36] and potentially break the chain of multi-organ failure from the very beginning. These were testified by our further findings, in which the biochemical parameters related to tissue damage and histological alterations in the pancreas, kidney, lung, and heart were all improved upon MSCs administration. In particular, the kidney becomes fragile in the background of SAP. The incidence of SAP patients complicated with renal injury or failure is reported to be as high as 50 % [37], and the mortality rate in these patients can range up to 74.7 % [38]. Prominent renal inflammation along with kidney injury was also induced in our SAP model, which amazingly, almost vanished soon after MSCs treatment, accompanied by a drastic decrease of serum creatinine and blood urea nitrogen as well as renal tissue inflammation. The lung is another vulnerable organ in SAP. Respiratory failure is the commonest OF in SAP [25,39]. Despite new understanding of the pathogenesis, diagnosis, and treatment of SAP-related ARDS, the drugs on clinical trials have failed to improve the prognosis of SAP-related ARDS patients [40]. Lung injury was rapidly induced in our SAP model. We found robust evidence on transcription, protein, and histological level that the infusion of MSCs significantly alleviated inflammation, tissue damage, lung edema, and neutrophil infiltration, which could potentially prevent the progression of acute lung injury to ARDS. Respiratory and renal failures are quite similar in their impact on the outcome, but

cardiovascular failure leads to the worst [25]. However, the SAP-related cardiac injury is characterized by cardiac insufficiency or dysfunction and decreased mean arterial pressure without histological injury. Similarly, a mild cardiovascular dysfunction with marked change on levels of inflammatory factors was induced in our SAP model, which was also significantly suppressed by the administration of MSCs. To our knowledge, this is the first research to investigate the effect of MSCs on renal and cardiac injuries induced by SAP. Our results provided sound and reliable evidence that MSCs could inhibit local and systemic inflammation and retard, even reverse the progression of renal, respiratory, and cardiovascular failure simultaneously.

As rare previous studies have investigated the effect of MSCs on multiple OF in SAP, let alone the optimal MSCs origin. UC-MSCs and BMSCs are the most commonly used and easily accessible origins, and are therefore compared in the current study [41]. We found that although they both effectively attenuated systemic and tissue inflammatory responses, UC-MSCs showed superior treatment effect in our SAP model. H. Wegmeyer et al. found that UC-MSCs showed a higher secretion of hematopoietic growth factor, macrophage-stimulating factor, and MCP-1 than BMSCs, these factors are involved in the differentiation of dendritic cells, induction of Tregs, and inhibition of T cell proliferation, this indicated that UC-MSCs have stronger immunomodulation capacity [14]. As inflammation is the core pathophysiologic procedure in SAP-related OF, this explains, to a certain extent, the superior efficacy of UC-MSCs in treating SAP. Consistently, previous studies found that UC-MSCs express more stemness and growth-related genes than BMSCs [42], and UC-MSCs are able to secrete more abundant angiogenic factors, which are more effective in inducing endothelial cell migration and micro vessel generation [43]. Our research provides a theoretical basis for selecting the MSCs source in SAP treatment.

Enhanced homing of MSCs to various organs have been reported in SAP models compared to normal control, with some discrepancies among researches. Garg PK. et al. Observed enhanced recruitment of human bone marrow-derived clonal MSCs (hcMSCs) to the spleen in cerulein and lipopolysaccharide induced SAP rat model, while labeled hcMSCs could not be detected in other organs [5]. Mofidi R et al. reported primary engraftment of hcMSCs to the injured pancreas, far more than to the lung, liver and spleen in 3 % sodium taurocholate induced SAP rat model [4]. While Foster BR et al. found that hcMSCs were primarily trapped in the lungs, with limited migration to the pancreas, the heart and the kidney [27]. In our study, we found tremendous engraftment of UC-MSCs to the lung ($P < 0.05$, Fig. 7E–F), with less but still considerable homing to the pancreas ($P < 0.05$, Fig. 7A–B) and kidney ($P < 0.05$, Fig. 7C–D). Migration of UC-MSCs to the cardiac tissue was hardly detected, consistent with previous reports. Such discrepancies might be attributed to the origins of MSCs, different SAP model establishments, and different animals.

The exact pathophysiologic mechanisms underlying SAP related multi-organ damage remain unknown. Macrophages playing a crucial role in SAP pathogenesis might be a popular opinion, with M1-type macrophages being the primary immune cell infiltrating in the early stages of AP. Pancreatic alveolar cells release danger-associated molecular patterns (DAMPs), triggering the polarization of macrophages towards the pro-inflammatory M1 type and the subsequent release of TNF-α, IL-1β, IL-6, IL-18, and MCP-1. These factors recruit additional macrophages, exacerbating local pancreatic inflammation. Simultaneously, circulating inflammatory cytokines activate macrophages in remote organs, leading to multi-organ failure [5,44]. Inflammation causes endothelial damage, coagulation system activation, and increased capillary permeability, resulting in inadequate circulating blood volume, which is the potential hazard of [5,45]. MSCs have been shown to convert pro-inflammatory M1 type macrophages into anti-inflammatory M2 type macrophages in various disease models, thereby reducing the inflammatory state and improving organ function. Moreover, Tan JH et al. observed notable structural alterations in organelles including the endoplasmic reticulum (ER), mitochondria, and nucleus in various injured organs as well as pancreas during SAP, such as the lung, liver, and kidney [46]. The role of mitochondrial dysfunction and ER stress in the pathogenesis of in SAP appeared crucial and should be further investigated. Our study has certain limitations. First, we compared the treatment effect of MSCs from the umbilical cord and bone marrow, yet the sources of MSCs are diverse, and the optimal treatment choice remains to be determined. Second, we only showed a stunning treatment effect of MSCs in mitigating OF in SAP, further exploration would be needed to unravel the precise mechanisms. Accordingly, our team is working on whether MSCs improve OF by remodeling the M1/M2 ratio in different organs, as well as the role of mitochondrial transfer in the onset and progression of diseases.

In conclusion, we demonstrated that MSCs infusion significantly mitigated pancreatic injury and multiorgan impairment in SAP, with UC-MSCs exhibiting superior efficacy compared with BMSCs. UC-MSCs may represent an optimal choice for MSCs-based treatment in SAP patients.

Funding

Ethics approval and consent to participate

The project was approved by the medical ethics committee of the Chinese PLA General Hospital, Medical School of Chinese PLA, the ethics approval number is SQ2022473.

Data Availability

Data could be obtained upon request to the corresponding author.

CRediT authorship contribution statement

Rui Ren: Writing – original draft, Software, Resources, Investigation, Formal analysis. **Weizheng Ren:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Data curation. **Yue Zhang:** Writing – original draft, Software, Resources, Investigation, Formal analysis. **Haixia Zhang:** Visualization, Supervision, Software, Project administration, Data curation. **Wanlu Su:** Visualization, Supervision, Software, Resources, Project administration, Data curation. **Ruofan Hu:** Validation, Supervision, Resources, Project administration, Methodology, Data curation. **Jian Zhao:** Resources, Formal analysis, Data curation. **Lei He:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition. **Yiming Mu:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition. **Yu Cheng:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35785.

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