



Physiological Modulation of Neutrophil Activation and Recruitment by Heat Shock Protein 90 in Acute Pancreatitis

^{1,4,5}Mohammed Merza, ²Huda Jabbar Jawhar, ³Zhian Sherzad Hayder, ⁶Kewan Kamal Ahmad, ⁷Roza Talaat Yaseen

¹College of Pharmacy, Hawler Medical University, Kurdistan Region, Iraq.

²Department of Pharmacy, College of Pharmacy, Knowledge University, Erbil, Iraq

³College of Dentistry, Hawler Medical University, Kurdistan Region, Iraq.

⁴Department of Pharmacy, College of Pharmacy, Knowledge University, Erbil 44001, Iraq

⁵Department of Pharmacy, College of Pharmacy, Catholic University in Erbil, Erbil, Iraq

⁶Department of Biology, Faculty of Science and Health, Koya University, Koya KOY45, Kurdistan Region- F.R. Iraq.

⁷Department of Nursing, Faculty of Nursing, Tishk International University

*Correspondence and request for reprints to:

Mohammed Yousif Merza, BSc, MSc, PhD
Department of Clinical Analysis, College of Pharmacy
Hawler Medical University-Erbil, Kurdistan, Iraq

Abstract

Background and Objective: Severe acute pancreatitis (AP) is characterized by leukocyte infiltration and tissue necrosis, yet the cellular signaling pathways driving pancreatic damage remain poorly understood. Heat shock protein 90 (HSP90) is a key regulator of specific cellular functions, promoting pro-inflammatory responses. This study investigates the role of HSP90 signaling in acute pancreatitis.

Methods: Acute pancreatitis was induced in C57BL/6 mice through taurocholate infusion into the pancreatic duct. Prior to pancreatitis induction, the animals received radicicol (60 mg/kg) as an HSP90 inhibitor.

Results: Radicicol administration significantly reduced taurocholate-induced increases in serum amylase, pancreatic neutrophil infiltration, acinar cell necrosis, and edema, which were associated with lung injury. Furthermore, inhibiting HSP90 decreased MPO levels in both the pancreas and lungs in response to taurocholate exposure. However, radicicol treatment did not affect the formation of macrophage inflammatory protein-2 (MIP-2) in the pancreas. Notably, HSP90 inhibition suppressed Mac-1 expression in neutrophils of mice with pancreatitis. Additionally, radicicol significantly reduced MIP-2-induced Mac-1 upregulation in isolated neutrophils in vitro, indicating a direct role of HSP90 in regulating Mac-1 expression. Finally, HSP90 inhibition had no direct effect on secretagogue-induced trypsinogen activation in pancreatic acinar cells in vitro.

Conclusion: These findings highlight the critical role of HSP90 signaling in acute pancreatitis, particularly in modulating neutrophil infiltration and tissue damage through Mac-1 expression. Beyond providing insights into the signaling mechanisms underlying pancreatitis and lung injury, these results suggest that HSP90 may serve as a potential therapeutic target.

protein might constitute a novel target in the management of severe AP related with lung injury.

Key words: Amylase, Chemokines, Inflammation, Leukocytes, and Pancreas



Introduction

Acute pancreatitis (AP) varies significantly in severity, ranging from mild, transient pain to serious local and systemic complications (1). Due to a limited understanding of the underlying pathophysiology, managing severe AP remains a significant challenge for clinicians, primarily relying on supportive care. Currently, there is no reliable method to predict the severity and outcomes of AP. Research indicates that trypsinogen activation, inflammation, and impaired microvascular perfusion are interconnected factors in the pathophysiology of pancreatitis (2). Given that trypsinogen activation is an early and transient event, the persistent inflammation in the pancreas may serve as a more logical target for treatment. The accumulation of leukocytes is a defining feature of inflammation, and numerous studies have highlighted their critical role in the progression of AP (3). The process of leukocyte extravasation involves several sequential steps mediated by specific adhesion molecules, including P-selectin, Mac-1, and LFA-1. The movement of leukocytes through tissues is guided by secreted chemokines. CXC chemokines, like macrophage inflammatory protein-2 (MIP-2), facilitate the extravascular recruitment of neutrophils. CXCR2 acts as the high-affinity receptor for MIP-2 and KC on murine neutrophils, and it has been shown to be essential for neutrophil infiltration in the pancreas (4). Although the roles of specific adhesion molecules and chemoattractants in leukocyte infiltration are relatively well understood, the signaling pathways that coordinate pro-inflammatory responses in AP are still not fully elucidated.

Trauma and infection activate various signaling pathways that converge on specific transcription factors, regulating the expression of pro-inflammatory genes. This signaling process is predominantly governed by intracellular kinases that phosphorylate downstream targets. Targeting HSP90 has been shown to protect against intestinal inflammation and leakage, potentially providing a beneficial strategy for alleviating intestinal failure in polymicrobial sepsis. In several disease models, including pancreatitis, heat shock proteins (HSPs) function as



chaperone proteins that safeguard living cells from injury-inducing stimuli. However, dysregulated expression of HSPs has been observed in various disease conditions, including cancer (5). In pancreatic cancer cells, inhibiting HSP, particularly HSP70, induces caspase-dependent apoptotic cell death, representing a novel therapeutic approach for pancreatic cancer.

Considering these factors, we hypothesized that heat shock protein signaling could be implicated in severe AP. To investigate this, we utilized an experimental model of severe AP with lung injury in mice induced by retrograde infusion of taurocholate into the pancreatic duct, while also intervening with HSP90 activity through the administration of radicicol.



Materials and Methods

Animals

All experiments were conducted using male C57BL/6 mice weighing 20-25 g (6 to 8 weeks), obtained from Taconic. The animals were housed under a 12-hour light/dark cycle at 22°C and were provided with ad libitum access to water and standard chow. Mice were anesthetized through intraperitoneal (i.p.) injection of 75 mg/kg of ketamine hydrochloride (Hoffman-La Roche, Basel, Switzerland) and 25 mg/kg of xylazine (Janssen Pharmaceuticals, Beerse, Belgium) dissolved in 200 µl saline. Post-anesthesia analgesia was administered via subcutaneous injection of buprenorphine hydrochloride at a dose of 0.1 mg/kg (Schering-Plough Corporation, New Jersey, USA).

Animal Model of Acute Pancreatitis

After anesthetization, the mice underwent a midline laparotomy, and the second part of the duodenum and the papilla of Vater were identified. Traction sutures (7–0 prolene) were placed 1 cm from the papilla. A small puncture was made through the duodenal wall with a 23 G needle, and a non-radiopaque polyethylene catheter (ID 0.28 mm) was inserted through the puncture and advanced 1 mm into the common bile duct, connected to a micro-infusion pump (CMA/100, Carnegie Medicin, Stockholm, Sweden). The common hepatic duct was clamped at the liver hilum. A retrograde infusion of 10 µl of 5% sodium taurocholate (Sigma, St. Louis, MO, USA) was delivered into the pancreatic duct for 10 minutes. After the infusion, the catheter was removed, and the clamp on the common hepatic duct was released. The duodenal puncture was closed with a purse-string suture (7–0 monofilament), and the abdomen was closed in two layers after removing the traction sutures. The animals were allowed to recover and had free access to food and water. Prior to bile duct cannulation, mice received i.p. administration of radicicol (60 mg/kg, Sigma) or a vehicle (phosphate-buffered saline [PBS]). This dosage and administration



schedule for the HSP90 inhibitor were based on prior studies. Animals exposed to taurocholate were treated with either vehicle (n = 8) or radicicol (n = 8). Control mice undergoing laparotomy and sodium chloride infusion into the pancreatic duct were pretreated with vehicle (Sham, n = 8). In separate experiments, mice exposed to taurocholate were treated with vehicle (n = 8) or radicicol (n = 8) two hours after pancreatitis induction. All animals were euthanized 24 hours post-induction, and various parameters were assessed. Blood was collected from the tail vein for systemic leukocyte differential counts, and samples were taken from the inferior vena cava to measure serum amylase levels. Pancreatic tissue was collected and divided into two parts; one was snap-frozen in liquid nitrogen for biochemical analysis of myeloperoxidase (MPO) and MIP-2, while the other was fixed in formalin for histological examination. Lung tissue was also harvested for MPO measurements.

Amylase Measurements

Serum amylase was quantified using a commercially available assay (Reflotron®, Roche Diagnostics GmbH, Mannheim, Germany).

Systemic Leukocyte Counts

Blood from the tail vein was mixed with Turks solution (0.2 mg gentian violet in 1 ml glacial acetic acid, 6.25% v/v) at a 1:20 dilution. Leukocytes were categorized as mononuclear or polymorphonuclear cells using a Bürker chamber.

MPO Activity

All frozen pancreatic and lung tissues were pre-weighed and homogenized in a 1 ml mixture (4:1) of PBS and aprotinin (10,000 KIE/ml, Trasylol®, Bayer HealthCare AG, Leverkusen, Germany) for 1 minute. The homogenates were centrifuged (15,339×g, 10 minutes), and the



supernatants were stored at -20°C . The pellets were used for MPO assays as previously described. Each pellet was mixed with 1 ml of 0.5% hexadecyltrimethylammonium bromide, then frozen for 24 hours, thawed, sonicated for 90 seconds, and placed in a water bath at 60°C for 2 hours. MPO activity in the supernatants was measured spectrophotometrically by observing the change in absorbance during the MPO-catalyzed reaction with H_2O_2 at 450 nm, using a reference filter at 540 nm at 25°C . Results are expressed as MPO units per gram of tissue.

Tissue Preparation

Pancreatic samples were fixed in 4% formaldehyde phosphate buffer overnight, dehydrated, and embedded in paraffin. Six-micrometer sections were stained with hematoxylin and eosin and examined using light microscopy. The severity of pancreatitis was evaluated blindly using a previously established scoring system, assessing edema, acinar cell necrosis, hemorrhage, and neutrophil infiltration on a scale from 0 (absent) to 4 (extensive).

MIP-2 Levels

MIP-2 levels in serum and pancreatic tissues were determined from stored supernatants of homogenized pancreatic samples using double-antibody Quantikine enzyme-linked immunosorbent assay kits (R&D Systems Europe, Abingdon, UK) with recombinant murine MIP-2 as the standard. The minimum detectable protein concentration was less than 0.5 pg/ml.

Flow Cytometry Assay

Blood was collected (1:10 acid citrate dextrose) from both vehicle and pancreatitis mice. To prevent non-specific labeling, samples were incubated with anti-CD16/CD32 for 5 minutes before staining with a PE-conjugated anti-Gr-1 (clone RB6-8C5, eBioscience, San Diego, CA, USA) antibody. Erythrocytes were lysed, and the cells were fixed. Following centrifugation, cells were analyzed with a FACSCalibur flow cytometer (Becton Dickinson, Mountain View,



CA, USA). A viable gate was used to exclude dead and fragmented cells, and Mac-1 expression was assessed on Gr-1-positive cells, which are identified as neutrophils.

Trypsinogen Activation in Isolated Acinar Cells

Pancreatic acinar cells were isolated via collagenase digestion and gentle shearing as previously described. The cells were suspended in HEPES-Ringer buffer (pH 7.4) saturated with O₂ and passed through a 150 µm cell strainer (Partec, England). Isolated acinar cells (1×10^7 cells per well) were preincubated with vehicle or radicicol (200 µM, 30 minutes) and then stimulated with 100 nM cerulein (37°C, 30 minutes) in duplicate. The buffer was discarded, and the cells were washed twice with buffer (pH 6.5) containing 250 mM sucrose, 5 mM 3-(morpholino) propanesulfonic acid (MOPS), and 1 mM MgSO₄. The cells were then homogenized in cold (4°C) MOPS buffer using a Potter-Elvehjem-type glass homogenizer. The resulting homogenate was centrifuged (56×g, 5 minutes), and the supernatant was used for assay. Trypsin activity was measured fluorometrically using Boc-Glu-Ala-Arg-MCA as the substrate. A 200 µl aliquot of the acinar cell homogenate was mixed with assay buffer (50 mM Tris, 150 mM NaCl, 1 mM CaCl₂, and 0.1% BSA, pH 8.0). The reaction was initiated by adding the substrate, and fluorescence emitted at 440 nm in response to excitation at 380 nm was monitored. Trypsin levels (pg/ml) were calculated using a standard curve generated with purified trypsin. The viability of pancreatic acinar cells was greater than 95%, as determined by trypan blue dye exclusion.

Analysis of Data

Data are presented as mean values ± SEM. Statistical evaluations were conducted using non-parametric tests (Mann–Whitney). A p-value of < 0.05 was considered significant, with "n" representing the number of animals used in the experiments.



Results

Heat Shock Protein 90 Regulates Tissue Damage in Pancreatitis

To investigate the role of heat shock protein 90 in severe acute pancreatitis (AP), we initially measured serum amylase levels as a marker of tissue damage. Our results showed that retrograde infusion of taurocholate into the pancreatic duct led to a fivefold increase in serum amylase levels (Table 1). Treatment with the heat shock protein inhibitor radicicol reduced taurocholate-induced serum amylase levels from $400 \pm 0.4 \mu\text{Kat/l}$ to $200 \pm 0.22 \mu\text{Kat/l}$, 1, $P < 0.05$ compared to vehicle + taurocholate, $n = 6$).

Parameters	Mean	SE
Sham	74	± 3
Pancreatitis	400*	± 6
Anti-HSP90	200#	± 4

Table 1. Blood amylase levels ($\mu\text{Kat/l}$) were measured in sham and taurocholate-exposed mice that were pretreated with either PBS or the heat shock protein 90 inhibitor radicicol (60 mg/kg). Blood samples were collected 24 hours after the induction of pancreatitis. The data are presented as means $\pm$ SEM, with $n = 6$. Significant differences are indicated as # $P < 0.05$ compared to sham and * $P < 0.05$ compared to PBS + taurocholate.

Examination of tissue morphology showed that control mice exhibited normal pancreatic microarchitecture (Table 2, $n = 6$), while taurocholate exposure caused significant destruction of the pancreatic tissue, characterized by acinar cell necrosis, edema formation, and neutrophil



accumulation (Table, n = 6). Notably, the inhibition of heat shock protein 90 offered protection against the tissue damage induced by taurocholate (Table 2, n = 6). Specifically, treatment with radicicol resulted in a significant reduction in taurocholate-induced acinar cell necrosis and significantly reduction in edema in the pancreas (Table 2, P < 0.05 compared to vehicle + taurocholate, n = 6). Additionally, radicicol decreased the number of extravascular leukocytes in mice with pancreatitis (table 2 P < 0.05 compared to vehicle + taurocholate, n = 6). Taurocholate challenge led to an increase in circulating mononuclear leukocytes (MNLs) and polymorphonuclear leukocytes (PMNLs), indicating ongoing systemic activation (Table 3). Importantly, the inhibition of heat shock protein 90 normalized the differential leukocyte counts in circulation, returning them to levels observed in control animals (Table 3).

Parameters	Hemorrhage (Scores)	Acinar cell necrosis (Scores)	Edema formation (Scores)	Neutrophil infiltration (Scores)
Sham	0.6	1.8	1.6	1.0
Pancreatitis	3.5*	4*	3.0*	3.5*
Anti-HSP90	1.0 [#]	1.5 [#]	2 [#]	2 [#]

Table 2:. Samples were collected 24 hours after pancreatitis induction. Scale bars represent 100 μm. Data are presented as means ± SEM, with n = 8. Significant differences are indicated as [#]P < 0.05 compared to sham and *P < 0.05 compared to PBS + taurocholate.

Table 3. Systemic leukocyte differential counts

	PMNL	MNL
Sham	1.0 ± 0.6	8.6 ± 0.3
PBS + Pancreatitis	0.6 ± 0.8 [#]	5.2 ± 0.8 [#]
Anti HSP90 + Pancreatitis	1.2 ± 0.6	10.6 ± 0.2

3421



Table 5. MIP-2 levels in the pancreas (pg/mg)

Parameters	Mean	SE
Sham	42.8	± 6
Pancreatitis	236.8*	±8
Anti-HSP90	86.4 [#]	±6

Table 5: Chemokine formation in the pancreas. MIP-2 levels in the pancreas were assessed in sham and taurocholate-exposed mice pretreated with either PBS or the HSP 90 inhibitor (60 mg/kg). Samples were collected 24 hours after the induction of pancreatitis. Data are presented as means ± SEM, with n = 6. Significant differences are indicated as [#]P < 0.05 compared to sham and *P < 0.05 compared to PBS + taurocholate.

The administration of radicicol did not significantly affect MIP-2 levels in the inflamed pancreas (Table 5, P > 0.05 compared to vehicle + taurocholate, n = 6). Additionally, we observed that Mac-1 expression was elevated on the surface of neutrophils in mice with pancreatitis (Table 5, P < 0.05 compared to Sham, n = 6), indicating systemic activation in this experimental model. Inhibition of heat shock protein 90 signaling significantly reduced the expression of Mac-1 on neutrophils in pancreatitis (Table 5, P < 0.05 compared to vehicle + taurocholate, n = 6). To determine whether the inhibitory effect of radicicol on Mac-1 expression was direct or indirect, we stimulated isolated neutrophils with MIP-2 in vitro and found that radicicol substantially decreased MIP-2-induced upregulation of Mac-1 on these isolated neutrophils (Fig. 1, P < 0.05 compared to vehicle + taurocholate, n = 6).

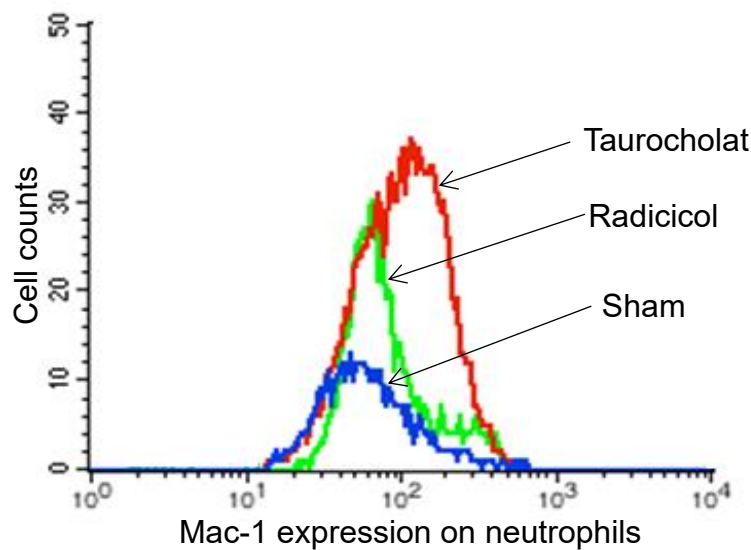


Figure 1. HSP 90 regulates Mac-1 expression on neutrophils. Mac-1 expression on neutrophils in PBS and radicicol (60 mg/kg) treated animals 24 h after induction of pancreatitis and (C+D) on isolated neutrophils incubated with MIP-2 (5 µg/ml) and PBS or radicicol (100 µM). Fluorescence intensity is shown on the x-axis and cell counts on the y-axis. Histograms are representative of 8 samples. Data represent means ± SEM and $n = 8$. $^{\#}P < 0.05$ versus sham and $^{*}P < 0.05$ versus PBS + taurocholate.

Trypsinogen Activation in Acinar Cells In Vitro

We further investigated whether heat shock protein 90 (HSP 90) might regulate trypsinogen activation in pancreatic acinar cells in vitro. Acinar cells were isolated from the pancreas of mice and incubated with cerulein. Our results showed that cerulein stimulation led to a more than 30-fold increase in trypsinogen activation compared to unstimulated cells (Fig. 2, $P < 0.05$ compared to control, $n = 5$). However, preincubation of the acinar cells with radicicol did not affect the secretagogue-induced activation of trypsinogen (Fig. 2, $P < 0.05$ compared to vehicle + cerulein, $n = 5$).

40 mini

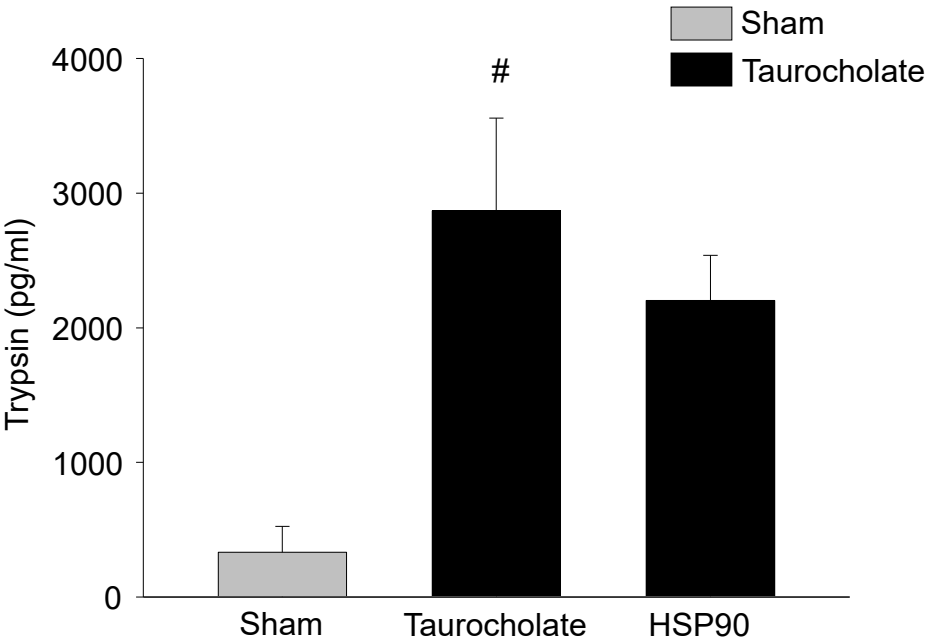


Figure 2. Measurement of Trypsinogen Activation in Acinar Cells
Trypsinogen activation was assessed in negative control cells and cerulein-exposed acinar cell homogenates pretreated with either PBS or radicicol (200 μ M). The activation of trypsinogen was quantified by measuring the enzymatic activity of trypsin fluorometrically, using Boc-Gln-Ala-Arg-MCA as the substrate, as detailed in the Materials and Methods section. Trypsin levels (pg/ml) were calculated based on a standard curve generated from assays of purified trypsin. Data are expressed as means $\pm$ SEM with n = 8. #P < 0.05 compared to PBS control.



Discussion

The signaling pathways that regulate pro-inflammatory processes in pancreatitis remain partially understood. Our study reveals, for the first time, that heat shock protein 90 (HSP 90) plays a crucial role in the pathophysiology of severe acute pancreatitis (AP). Specifically, we found that HSP 90 is involved in the upregulation of Mac-1 on neutrophils. Inhibiting HSP 90 activity not only reduces neutrophil infiltration in the pancreas but also mitigates acinar cell necrosis and serum amylase levels associated with AP. Furthermore, we observed that blocking HSP 90 signaling prevents neutrophil accumulation in the lungs, indicating that HSP 90 regulates both local and systemic inflammation in severe AP.

Previous studies have demonstrated that HSP 90 activity influences pro-inflammatory responses in models of multiple sclerosis and sepsis (5). In this study, we utilized a specific HSP 90 inhibitor, radicicol, which significantly decreased tissue damage in severe AP. Notably, radicicol treatment led to reduced levels of blood amylase and acinar cell necrosis provoked by taurocholate, suggesting that HSP 90 is critical for mediating tissue damage in severe AP. This provides the first evidence in literature that targeting the HSP 90 signaling pathway can offer protective effects against severe AP. Interestingly, heat shock proteins, often associated with regulating severe cardiovascular conditions, have also been found to mitigate experimental pancreatitis (6).

Neutrophil infiltration is a well-documented characteristic of pancreatitis. Neutrophil depletion has been shown to consistently improve tissue damage in AP (7). In our study, we found that taurocholate exposure significantly increased myeloperoxidase (MPO) activity and the number of extravascular neutrophils in the pancreas. Treatment with radicicol markedly reduced MPO levels and neutrophil counts, indicating that HSP 90 is a vital regulator of neutrophil recruitment in the inflamed pancreas. Given the critical role of neutrophils in pancreatitis, the inhibitory effects of radicicol on neutrophil responses may explain its protective impact in AP (8). Additionally, systemic complications of severe AP include pulmonary neutrophil accumulation, which we observed with increased lung MPO levels following taurocholate exposure. Radicicol treatment decreased MPO activity in the lungs, suggesting that HSP 90 also modulates systemic activation and infiltration of neutrophils during severe AP.



Specific adhesion molecules govern the extravasation of leukocytes, and while the role of certain adhesion molecules in pancreatic leukocyte accumulation remains unclear, Mac-1 has been identified as a key mediator in neutrophil tissue infiltration (9). We observed that taurocholate administration increased Mac-1 expression on neutrophils, and importantly, radicicol significantly reduced this expression. This suggests that HSP 90 regulates Mac-1 levels on neutrophils in the context of AP. We also explored whether radicicol's effects on Mac-1 expression might stem from its influence on CXC chemokines, particularly MIP-2, a potent neutrophil activator. Although taurocholate exposure increased pancreatic MIP-2 levels, radicicol did not alter this production, leading us to investigate whether radicicol could directly inhibit Mac-1 upregulation on activated neutrophils. Indeed, we found that radicicol effectively abolished MIP-2-induced Mac-1 expression on isolated neutrophils, indicating a direct regulatory role of HSP 90 on Mac-1 expression.

Trypsinogen activation is widely recognized as a pivotal aspect of AP pathophysiology (10). Previous research has indicated that GTPase signaling plays a role in trypsinogen activation in acinar cells (11,12). However, we found that inhibiting HSP 90 did not affect secretagogue-induced trypsin activation in vitro. This suggests that the protective effects of HSP 90 may be downstream of trypsin activation in pancreatitis. Our findings imply that the primary role of HSP 90 in AP is associated with inhibiting Mac-1 expression and subsequent neutrophil accumulation in both the pancreas and lungs.

In conclusion, our findings highlight that HSP 90 signaling is a critical regulator of tissue damage in severe AP. By inhibiting HSP 90, we observed a reduction in Mac-1 expression and neutrophil infiltration in both the pancreas and lungs, indicating that HSP 90 plays a significant role in managing local and systemic inflammation in pancreatitis. These results not only clarify a key signaling mechanism in AP but also suggest that targeting HSP 90 could be an effective strategy for mitigating pathological inflammation in severe AP.



Acknowledgements

Clinical Resrach Center, Faculty of Medicine, Surgery Unit. Hawler Medical Univeristy, College of Pharmacy, Clinical Analysis Department.



References

1. Liu X, Zheng Y, Meng Z, Wang H, Zhang Y, Xue D. Gene Regulation of Neutrophils Mediated Liver and Lung Injury through NETosis in Acute Pancreatitis. Inflammation. 2024.
2. Wetterholm E, Linders J, Merza M, Regner S, Thorlacius H. Platelet-derived CXCL4 regulates neutrophil infiltration and tissue damage in severe acute pancreatitis. Transl Res. 2016;176:105-18.
3. Dahiya DS, Jahagirdar V, Chandan S, Gangwani MK, Merza N, Ali H, et al. Acute pancreatitis in liver transplant hospitalizations: Identifying national trends, clinical outcomes and healthcare burden in the United States. World J Hepatol. 2023;15(6):797-812.
4. Merza M, Wetterholm E, Zhang S, Regner S, Thorlacius H. Inhibition of geranylgeranyltransferase attenuates neutrophil accumulation and tissue injury in severe acute pancreatitis. J Leukoc Biol. 2013;94(3):493-502.
5. Yuchen Zhang , Michael B Ware , Mohammad Y Zaidi , Amanda N Ruggieri , Brian M Olson , Hannah Komar , Matthew R Farren , Ganji Purnachandra Nagaraju , Chao Zhang , Zhengjia Chen , Juan M Sarmiento , Rafi Ahmed , Shishir K Maithel , Bassel F El-Rayes , Gregory B Lesinski. Heat Shock Protein-90 Inhibition Alters Activation of Pancreatic Stellate Cells and Enhances the Efficacy of PD-1 Blockade in Pancreatic Cancer. Mol Cancer Ther . 2021 Jan;20(1):150-160.
6. R T Ethridge, R A Ehlers, M R Hellmich, S Rajaraman, B M Evers . Acute pancreatitis results in induction of heat shock proteins 70 and 27 and heat shock factor-1. Pancreas. 2000 Oct;21(3):248-56.
7. Merza M, Rahman M, Zhang S, Hwaiz R, Regner S, Schmidtchen A, et al. Human thrombin-derived host defense peptides inhibit neutrophil recruitment and tissue injury in severe acute pancreatitis. Am J Physiol Gastrointest Liver Physiol. 2014;307(9):G914-21.
8. Carrion-Barbera I, Triginer L, Tio L, Perez-Garcia C, Ribes A, Abad V, et al. Serum Advanced Glycation End Products and Their Soluble Receptor as New Biomarkers in Systemic Lupus Erythematosus. Biomedicines. 2024;12(3).
9. Butcher L, Carnicero JA, Peres K, Bandinelli S, Garcia-Garcia FJ, Rodriguez-Artalejo F, et al. Frailty Influences the Relationship between the Soluble



Receptor for Advanced Glycation-End Products and Mortality in Older Adults with Diabetes Mellitus. *Gerontology*. 2024;70(6):585-94.

10. Yang J, Wei A, Wu B, Deng J. Predictive value of combination of lung injury prediction score and receptor for advanced glycation end-products for the occurrence of acute respiratory distress syndrome. *Exp Ther Med*. 2024;27(1):4.

11. Robin H, Trudeau C, Robbins A, Chung E, Rahman E, Gangmark-Strickland O, et al. A Potential Role for the Receptor for Advanced Glycation End-Products (RAGE) in the Development of Secondhand Smoke-Induced Chronic Sinusitis. *Curr Issues Mol Biol*. 2024;46(1):729-40.

12. Merza M, Hartman H, Rahman M, Hwaiz R, Zhang E, Renstrom E, et al. Neutrophil Extracellular Traps Induce Trypsin Activation, Inflammation, and Tissue Damage in Mice With Severe Acute Pancreatitis. *Gastroenterology*. 2015;149(7):1920-31 e8.