Rac-1 Inhibitor Attenuates Neutrophil Infiltration and Tissue Damage in Severe Acute Pancreatitis

Helen Jawdat Sabri[1], Mohammed Yousif Merza [1,2]
[1]Department of Medical Biochemical Analysis, Cihan University-Erbil, Kurdistan Region, Iraq.
[1,2]Department of Clinical Analysis, College of Pharmacy, Hawler Medical University, Erbil, Kurdistan Region of Iraq.

Abstract— **Background: Acute pancreatitis is a common inflammatory disease of the exocrine pancreas, characterized by a mortality rate ranging from 1% to 5%. This condition has the potential to lead to organ dysfunction, pancreatic necrosis, and subsequent organ failure. Aim: Rac1, a G-protein with a molecular weight of approximately 21 kD, has been demonstrated to govern various platelet functions. We hypothesized that the inhibition of Rac-1 signaling could be implicated in severe acute pancreatitis (AP). Our study aimed to explore the impact of a Rac-1 inhibitor on neutrophil release and its potential in safeguarding against tissue damage associated with severe acute pancreatitis. Method: In this experimental study, Swiss albino mice were employed, and the mice received pretreatment with a specific Rac1 inhibitor, NSC23766, at a dosage of 5 mg/kg. Subsequently, a midline laparotomy was performed on the anesthetized animals, followed by taurocholate perfusion. Blood amylase levels were measured, and the concentration of serum chemokines (CXCL2 and IL6) was assessed using ELISA. Additionally, leukocyte counting, myeloperoxidase (MPO) activity, and histopathological examination were conducted to analyze morphological changes in the pancreas. Results: The blood amylase levels were notably elevated in mice with acute pancreatitis compared to the sham mice. Our findings indicated that acute pancreatitis was induced in mice through taurocholate perfusion. Rac1 expression was upregulated in mice with acute pancreatitis, and pre-treatment with NSC23766 led to a significant decrease in myeloperoxidase (MPO) levels compared to the acute pancreatitis group. Furthermore, the levels of CXCL2 and IL6 were significantly higher in acute pancreatitis compared to the control group, with a P-value < 0.05. Conclusion: Our investigation underscores the pivotal role of Rac-1 in regulating amylase activity during severe acute pancreatitis (AP). Additionally, it highlights the involvement of Rac-1 in platelet activation and the secretion of chemokines, namely CXCL2 and IL6, attributed to inflammation in organ tissues during acute pancreatitis. Targeting Rac1 emerges as a promising and innovative approach for the treatment of severe acute pancreatitis, offering potential control over inflammation and tissue damage.**

Index Terms— **Rac-1, Chemokines, acute pancreatitis, Inflammation, and leukocyte.**

I. Introduction

Acute pancreatitis is an exocrine frequent inflammatory disease with a mortality rate between (1-5) %. Which can cause dysfunction of multiple organs, consequences pancreatic necrosis and the failure of the organs [1]. Two distinct phases have been identified in the progression of acute pancreatitis: an early and late phase. The severity of the condition is categorized as mild, moderate, or severe to diagnose acute pancreatitis. At least two of the specified symptoms must be evident: Enduring and intense pain in the upper central abdomen, often extending to the back, accompanied by a sudden onset, and elevated levels of serum lipase or amylase activity, surpassing at least three times the upper limit of normal [2]. Polymorphonuclear (PMN) leukocytes, also known as neutrophils are essential for the host's defense against fungal, bacterial, and viral infections. Additionally, neutrophils play a part in a number of chronic illnesses, including autoimmune disorders, cancer, atherosclerosis, and allergies [1], [3]. Neutrophils are predominantly produced in the bone marrow of the adult mammals and discharged into the bloodstream, A sequence of actions occurs to recruit circulating neutrophils into the tissue, including crawling, vessel wall rolling on, arrest, and transmigration. As a result of chemotactic clues, neutrophils migrate during transmigration from the lumen vessel toward the inflammatory tissue [4]. The onset of acute pancreatitis (AP) arises from the activation of trypsin within the pancreas, leading to self-digestion of pancreatic tissue, accompanied by edema, hemorrhages, necrosis, and inflammation. Currently, the complete understanding of the pathophysiology of acute pancreatitis remains elusive. However, key concepts include self-digestion of the pancreas, elevated calcium levels in pancreatic cells, the presence of inflammatory mediators, apoptosis, and bacterial translocation from the intestine. Pancreatic acinar cells, rich in endoplasmic reticulum (ER), adjust to their physiological role of producing digestive enzymes. This involves the synthesis of various new digestive enzymes within the ER. The coordination of protein folding and post-translational modifications is facilitated by the collaborative action of BiP, ATP enzymatic activity, and the energy-consuming process of ATP hydrolysis. Furthermore, the Golgi apparatus aids in packaging, ultimately resulting in the secretion of zymogen granules extracellularly [5]. Rac1, a member of the Rho family, is a small GTPase that functions as a molecular switch, playing a role in the regulation of various essential cellular functions. [6]. The signal transducer Rac-1, expressed widely, oversees various activities associated with

DOI: http://doi.org/10.24086/biohs2024/paper.1359

inflammatory responses, including cell adhesion, chemotaxis, and vascular permeability regulation [7]. Several research studies have emphasized the involvement of Rac1 in conditions like sepsis, hyperglycemia, lamellipodia formation, phospholipase activation, granule secretion, and platelet clot retraction. However, the potential impact of a Rac-1 inhibitor in mitigating neutrophil infiltration and tissue damage in acute pancreatitis remains unexplored. Given the aforementioned considerations, our hypothesis was that the inhibition of Rac-1 signaling could be significant in severe acute pancreatitis (AP). Therefore, the primary objective of this study was to explore the impact of a Rac-1 inhibitor on neutrophil release and the protection against tissue damage caused by severe acute pancreatitis.

II. MATERIALS AND METHODS

A. Animals

Swiss albino male mice, with a weight ranging from 20 to 25 grams, were accommodated in a laboratory setting where they experienced a 12-hour light/12-hour dark cycle and were free access to both water and food. Permission for all experimental protocols was granted by the ethical committee of Hawler Medical University-College of Pharmacy. The mice were subjected to intraperitoneal (i.p.) anesthesia, with a dose of 25 mg/kg xylazine (Janssen Pharmaceutica, Beerse, Belgium) and 75 mg/kg ketamine hydrochloride (Hoffman-La Roche, Basel, Switzerland).

B. Taurocholate perfusion

A midline laparotomy was carried out on anesthetized animals, during which the vater papilla and the second portion of the duodenum were recognized. A 23-gauge needle was employed to create a small opening in the duodenum wall alongside the vater papilla, Traction sutures were positioned one centimeter from the papilla, and a polyethylene catheter linked to a micro-infusion pump (CMA/100, Carnegie Medicin, Stockholm, Sweden) was introduced into the common bile duct through a perforation in the duodenum, with a distance of one millimeter. The liver hilum was compressed, leading to the formation of the hepatic duct. A 5% solution of sodium taurocholate (Sigma, St. Louis, MO, USA), totaling ten microliters, was introduced into the pancreatic duct and left in place for ten minutes. After concluding the process, both the catheter and the clamp on the hepatic duct were taken out. The traction sutures were taken out, the puncture in the duodenum was sealed using a purse-string suture, and the abdominal closure was carried out in two layers. The mice are currently permitted to regain consciousness and have unrestricted access to food and water. Either the vehicle or the antibodies targeting RAC1, obtained from Abcam (Cambridge, MA), were intraperitoneally administered at a dosage of (5mg/kg) prior to bile duct cannulation. The dosage and the intraperitoneal administration of RAC1 inhibitors were adopted from a prior study [8]. Control mice, identified as sham mice, were administered intraperitoneal injections of phosphate-buffered saline (PBS) and underwent laparotomy. Subsequently, their pancreatic ducts were infused with the mixture (PBS, n = 6). Assessment of all parameters relevant to this study was conducted 24 hours after inducing pancreatitis in the mice.

C. Amylase enzyme activity

The levels of amylase enzyme activity were measured in blood extracted from a vein in the mouse tail using a commercially accessible assay (Reflotron®, Roche Diagnostics GmbH, Mannheim, Germany).

D. Examination of the pancreas's morphology

The pancreatic tissue was fixed in a 4% formaldehyde phosphate buffer, followed by dehydration and embedding in paraffin. The embedded material was then cut into six-micrometer slices, stained with hematoxylin and eosin, and observed under a light microscope. Employing the scoring system established earlier, which took into account edema, acinar cell necrosis, bleeding, and neutrophil infiltration on a scale from 0 (absent) to 4 (severe), the severity of pancreatitis was assessed in a blinded manner [9].

III. COUNTING THE LEUKOCYTES

Differential counts of systemic blood were conducted using blood extracted from the tail vein. Turks solution (Merck, Darmstadt, Germany) was diluted with the blood at a ratio of 1:20. Leukocytes were identified as mononuclear and polymorphonuclear cells using a Burker chamber.

A. Activity of myeloperoxidase (MPO)

To evaluate myeloperoxidase (MPO), small portions of lung and pancreatic (pancreatic head) tissues were collected. Before freezing, the tissues were weighed and homogenized in a 1 ml mixture (4:1 ratio) of PBS and aprotinin (10,000 KIE per ml; Trasylol®, Bayer HealthCare AG, Leverkusen, Germany) for a duration of 1 minute. After homogenization, the samples were centrifuged for 10 minutes at 15,339×g, and the resultant supernatant was preserved at −20°C for future utilization in the MPO assay. Subsequently, 1 milliliter of 0.5% hexadecyltrimethylammonium bromide was added to all of the pellets. Afterward, the sample was frozen for 24 hours, followed by thawing, sonication for 90 seconds, and then incubation for two hours at 60°C in a water bath. Subsequently, the assessment of MPO in the supernatant was performed using a spectrophotometric method. Enzyme activity was determined by measuring the changes in absorbance in the MPO-catalyzed H2O2 redox reaction at 450 nm (with a reference filter at 540 nm, 25°C), and the results were expressed in MPO units per gram of tissue.

B. Enzyme-linked immunosorbent assay (ELISA)

The levels of CXCL2 in pancreatic tissue and the plasma concentration of IL-6 were assessed using double-antibody quantifying enzyme-linked immunosorbent assay (ELISA) kits obtained from R & D Systems Europe, Abingdon, UK, the analysis utilized recombinant murine CXCL2 and IL-6 as

DOI: http://doi.org/10.24086/biohs2024/paper.1359

standards, assay kits have a sensitivity that can detect protein concentrations as low as less than 0.5 pg/ml.

C. Statistical analysis

The statistical analysis of the data was conducted using the Mann-Whitney test because it was non-parametric. The number of animals represented by "n" and the significant level, which was set at $P < 0.05$, were also determined. Additionally, the information displayed as mean values ± SEM.

IV. RESULTS

A. Rac-1 inhibitor regulates amylase activation in AP

To investigate the impact of a Rac-1 inhibitor on severe acute pancreatitis (AP) and its ability to regulate tissue damage, the study initially assessed blood amylase levels as a marker for tissue damage. Our findings revealed a 5.4-fold rise in amylase levels through retrograde taurocholate infusion into the pancreatic duct (Table 1, $P < 0.05$ vs. Sham, n = 6). Pre-treatment with the Rac-1 inhibitor led to a significant decrease in taurocholate-induced blood amylase levels, dropping from ±8.0 µKat/l to ±2.0 µKat/l, indicating a 65.6% reduction (Table 1, $P < 0.05$ vs. vehicle + taurocholate, n = 6).

Table 1. Blood amylase levels (expressed in µKat/L) were measured in sham mice and mice exposed to taurocholate, both groups receiving pretreatment with either a vehicle or the Rac-1 inhibitor (5mg/kg).

Parameters	Mean	SE
Sham	121	±3
Taurocholate	664*	±8
Anti-Rac1	436#	±2

Blood samples were obtained 24 hours following the onset of pancreatitis. The provided data represent means ± SEM, with a sample size of n = 6. *P < 0.05 in comparison to PBS, and #P < 0.05 in comparison to Vehicle + Taurocholate.

B. Histopathology

Analysis of tissue morphology indicated that in control mice (n = 6), the pancreatic microarchitecture was normal (Table 2). Conversely, exposure to taurocholate led to significant harm to the structural integrity of the pancreatic tissue, featuring hemorrhage, formation of edema, necrosis of acinar cell, and the accumulation of neutrophils (Table 2, n = 6). We discovered that the inhibition of Rac-1 provided protection against tissue damage induced by taurocholate (Table 2, n = 6). Specifically, the administration of Rac-1 was associated with a 42.8% reduction in taurocholate-induced hemorrhage, a 25.0% decrease in acinar cell necrosis, and a 28.5% reduction in pancreatic edema (Table 2, $P < 0.05$ vs. vehicle + taurocholate, n = 6). Moreover, Rac-1 caused a 33.3% reduction in the count of extravascular leukocytes in mice experiencing pancreatitis (Table 2, $P < 0.05$ vs. vehicle + taurocholate, n = 6).

Table 2. Rac1 modulates tissue damage in acute pancreatitis (AP)

Parameters	Necrosis of acinar cell (Scores)	Hemorrhage (Scores)	Neutrophil infiltration (Scores)	formation of edema (Scores)
Sham	1.0	0.3	1.0	0.8
Taurocholate	4.0*	3.5*	3.0*	3.5*
Anti-RAC1 +Taurocholate	1.0#	1.5#	1.0#	1.0#

Necrosis of acinar cell, hemorrhage, neutrophil infiltration, and formation of edema were evaluated in sham (PBS) animals and mice subjected to taurocholate with pretreatment of either vehicle or the Rac1 inhibitor (5mg/kg). Tissue samples were gathered 24 hours after pancreatitis induction. The provided data represent means ± SEM with a sample size of n = 6. *P < 0.05 compared to PBS, and #P < 0.05 compared to Vehicle + Taurocholate.

C. Rac-1 effects on leukocyte differential count in AP

Exposure to taurocholate resulted in an elevated count of circulating mononuclear leukocytes (MNLs) and polymorphonuclear leukocytes (PMNLs), indicating ongoing systemic activation (Table 3). The inhibition of Rac-1, however, reversed alterations in leukocyte differential counts in the circulation, bringing them back to levels observed in control animals (Table 3).

Table 3. Differential leukocyte counts in the systemic circulation

	MNL	PMNL	Total
PBS	12.1 ± 0.4	1.1 ± 0.8	13.9 ± 1.2
Taurocholate	4.5 ± 0.2*	0.4 ± 0.3*	4.9 ± 0.6*
Anti-RAC1 + Taurocholate	7.8 ± 0.2#	0.8 ± 0.6#	8.8 ± 0.8#

Blood samples were collected from mice in the sham group and those exposed to taurocholate, with preceding administration of either a vehicle or the Rac1 inhibitor (5mg/kg). Cells were classified as mono morphonuclear leukocytes (MNL) and polymorphonuclear leukocytes (PMNL). The provided data show the mean ± SEM, with a concentration of 106 cells/ml and a sample size of n = 6. #P < 0.05 in comparison to PBS, and *P < 0.05 in comparison to Vehicle + Taurocholate.

D. The Rac-1 inhibitor modulates the infiltration of neutrophils in pancreatitis

MPO levels were assessed in the pancreas (Table 4) and lung (Table 5) of sham mice and those exposed to taurocholate, with pretreatment using PBS or the Rac-1 inhibitor (5 mg/kg). Tissue MPO levels served as an indicator of neutrophil infiltration. Exposure to taurocholate led to a 12-fold increase in pancreatic MPO activity (Table 4, $P < 0.05$ vs. Sham, n = 6). The inhibition of Rac-1 resulted in a 37.3% reduction in taurocholate-induced pancreatic MPO levels (Table 4, $P < 0.05$ vs. vehicle + taurocholate, n = 6).

DOI: http://doi.org/10.24086/biohs2024/paper.1359

Table 4. Levels of myeloperoxidase (MPO) in the pancreas (expressed as U/g tissue)

Parameters	Mean	SE
Sham	0.40	± 0.2
Taurocholate	4.82*	± 0.3
Anti-Rac1+ Taurocholate	1.8#	± 1.1

RAC1 modulates the increase in neutrophils caused by taurocholate. MPO levels in the pancreas were examined in both sham (PBS) animals and mice subjected to taurocholate, with prior treatment involving either a vehicle or the Rac1 inhibitor (5mg/kg). Tissue samples were gathered 24 hours post pancreatitis induction. The provided data represent means ± SEM, with a sample size of n = 6. *P < 0.05 in comparison to PBS, and #P < 0.05 in comparison to Vehicle + Taurocholate.

In severe acute pancreatitis (AP), activated neutrophils accumulate in the pulmonary microvasculature as part of the systemic inflammatory response. Remarkably, the presence of taurocholate markedly elevated myeloperoxidase (MPO) activity in the lung. In mice subjected to taurocholate, the inhibition of Rac-1 resulted in a reduction of over 36.3% in MPO levels in the lung (Table 5, P < 0.05 vs. vehicle + taurocholate, n = 6).

Table 5. Levels of myeloperoxidase (MPO) in the lung (expressed as U/g tissue)

Parameters	Mean	SE
Sham	0.3	± 0.1
Taurocholate	6.32*	± 2.4
Rac-1+ Taurocholate	2.3#	± 0.8

Rac1 controls the build-up of neutrophils triggered by taurocholate. Myeloperoxidase (MPO) levels in the lung were assessed in sham (PBS) animals and mice exposed to taurocholate, with prior treatment involving either a vehicle or the Rac1 inhibitor (5mg/kg). Tissue specimens were gathered 24 hours post pancreatitis induction. The provided data represent means ± SEM, with a sample size of n = 5. *P < 0.05 in comparison to PBS, and #P < 0.05 in comparison to Vehicle + Taurocholate.

E. Inhibition of Rac-1 controls the levels of serum CXCL2 in acute pancreatitis (AP)

Additionally, it was noted that exposure to taurocholate significantly increased CXCL2 levels in the pancreas, escalating from 42.6 ± 1.2 to 144.3 ± 0.6 pg/mg, indicating a 3.3-fold rise (Table 6, P < 0.05 vs. Sham, n = 6). On the contrary, pre-administration of the Rac-1 inhibitor significantly decreased CXCL2 levels from 144.3 ± 0.6 to 88.2 ± 0.4 pg/mg, signifying a reduction of over 61% (Table 6, P < 0.05 vs. Vehicle + Taurocholate, n = 6).

Table 6. Levels of CXCL2 in the pancreas, (expressed as pg/mg)

Parameters	Mean	SE
Sham	42.6	± 1.2
Taurocholate	144.3*	± 0.6
Anti-Rac1+ taurocholate	88.2#	± 0.4

Levels of CXCL2 in the pancreas were assessed in both sham (PBS) animals and mice exposed to taurocholate, with prior treatment using either a vehicle or the Rac1 inhibitor (5mg/kg). Tissue specimens were gathered 24 hours after the initiation of pancreatitis. The provided data represent means ± SEM, with a sample size of n = 6. *P < 0.05 in comparison to PBS, and #P < 0.05 in comparison to Vehicle + Taurocholate.

F. Inhibition of Rac-1 controls plasma IL6 levels in acute pancreatitis (AP)

Acute pancreatitis increased P < 0.05 plasma levels of IL6 from 18.12 ± 1.6 pg/mg in sham mice up to 122 ± 6.2 pg/mg, corresponding to 15.0 -fold increase (Table 7). We found the induction of AP by Taurocholate suggesting that AP induces IL6 secretion, Notably Rac-1 inhibitor, significantly reduced P < 0.05 chemokine release brought by AP (Table 7). In AP pre treated with Rac-1 inhibitor reduced blood levels of IL6 from 122 ± 6.2 pg/mg to 88.4 ± 6.2 pg/mg, a decrease of more than 72.4 % (Table 6, P < 0.05 Vehicle+ Taurocholate, n = 6).

Table 7. Levels of IL6 in the plasma, (expressed as ng/ml)

Parameters	Mean	SE
Sham	8.12	± 1.6
Taurocholate	122*	± 6.2
Anti-RAC1	88.4#	± 6.2

Levels were assessed in sham (PBS) animals and mice subjected to taurocholate, with prior treatment involving either a vehicle or the RAC1 inhibitor (5mg/kg). Specimens were gathered 24 hours post pancreatitis induction. The provided data represent means ± SEM, with a sample size of n = 5. *P < 0.05 in comparison to PBS, and #P < 0.05 in comparison to Vehicle + Taurocholate.

DISCUSSION

The present study indicates that Rac-1-mediated neutrophil infiltration and tissue damage are crucial factors in severe acute pancreatitis. These results imply a pivotal role for neutrophils in the development of acute pancreatitis (AP). The Rac-1 inhibitor was employed to elucidate the involvement of Rac-1 in severe AP. The digesting enzyme amylase is mostly secreted by the pancreas. Amylase can rise quickly in acute pancreatitis can spike three to six hours after symptoms appear stay high for up to 5 days [10]. Earlier research has emphasized the crucial involvement of amylase in the initiation of acute pancreatitis. However, in our present study, we noted a significant rise in blood amylase levels after the retrograde infusion of taurocholate into the pancreatic duct in comparison to the sham group, with a P-value < 0.05. Consistent with our findings, prior

DOI: http://doi.org/10.24086/biohs2024/paper.1359

studies have also shown an increase in amylase levels following severe acute pancreatitis [11], [12], [13]. However, our investigation revealed that pretreatment with the Rac-1 inhibitor (5 mg·kg−1) led to a significant reduction in blood amylase, amounting to more than a 65.6% decrease (Table 1). This underscores the involvement of both amylase and Rac1 in acute pancreatitis (AP). Additionally, histological examination demonstrated substantial pathological changes in the pancreatic tissue of taurocholate-exposed mice, indicative of significant tissue damage. In our study, administration of the Rac-1 inhibitor (5 mg·kg−1) notably mitigated the extent of tissue damage (Table 2). Previous studies have also highlighted the role of pancreatic tissue damage in AP [14], [15], [16], [17]. Our results indicated that the Rac-1 inhibitor reversed alterations in leukocyte counts (Table 3). Yang et al. had previously noted the significance of neutrophil infiltration in severe acute pancreatitis [18]. Our findings align with this pattern, as demonstrated in (Table 4) and (Table 5), In this context, MPO levels act as an indicator of neutrophil infiltration. We noted a significant twelvefold rise in pancreatic MPO activity after exposure to taurocholate (Table 4). Interestingly, Pre-administration of the Rac-1 inhibitor markedly decreased taurocholate-induced MPO levels in both the pancreas and lungs by over 37% ($P < 0.05$). These findings align with those reported in earlier studies [19], [20]. The present results indicate the significance of Rac1-mediated platelet activation and the secretion of CXCL2 and IL6 derived from platelets in the context of acute pancreatitis (AP). The role of Rac1 in AP was explored using the Rac1 inhibitor NSC23766. Serum samples were collected from mice in sham, taurocholate-exposed, and Rac1 inhibitor pre-treated groups. It was observed that platelet reactivity is heightened in the presence of taurocholate induction. In agreement with this finding, better Rac-1 inhibitor has been linked to reduced platelet reactivity [21]. We discovered that mice exposure to taurocholate, increased platelet activity and chemokine secretion (CXCL2 and IL6). the level of CXCL2 in plasma from AP mice were 3.3-fold higher than in sham mice (Table 6). Remarkably, The Rac1 inhibitor (NSC23766) demonstrated a substantial decrease in the release of chemokines induced by acute pancreatitis (AP). The initiation of AP through taurocholate was effectively counteracted through the administration of the Rac1 inhibitor (5 mg/kg), resulting in a reduction of more than 61% (Table 6). Additionally, other research included the control of CXCL2, a platelet-derived chemokine, is involved in abdominal sepsis [22]. Interleukin-6 is a good marker for early diagnosis of the severe acute pancreatitis, in this research, we showed that AP markedly increased IL6 by 15- fold higher than control mice, moreover, we discovered that the Rac1 NSC23766 inhibition strongly decreased IL6 levels by more than 72.4%in mice induced with AP, (Table 7). Rac1 inhibitor may thus be a therapeutic agent for controlling AP by inhibiting chemokine, a signaling molecule in exaggerated inflammation.

Conclusion

In this investigation, we illustrated the regulatory role of Rac1 in severe acute pancreatitis (AP), particularly in modulating amylase activity. Additionally, we observed overexpression of the chemokines CXCL2 and IL6 in platelet activation among AP mice. The inhibitory effect of Rac-1 on neutrophil infiltration was also highlighted. Furthermore, the morphological changes associated with AP in mice were attributed to platelet activation. These findings suggest that Rac1 inhibition may serve as a therapeutic approach in regulating AP by mitigating the production of chemokines, key signaling molecules in inflammation, and subsequently reducing organ damage caused by activated platelets in AP.

References

[1] K. Ley et al., "Neutrophils: New insights and open questions," Sci. Immunol., vol. 3, no. 30, p. eaat4579, 2018.

[2] J. Walkowska, N. Zielinska, R. S. Tubbs, M. Podgórski, J. Dłubek-Ruxer, and Ł. Olewnik, "Diagnosis and treatment of acute pancreatitis," Diagnostics, vol. 12, no. 8, p. 1974, 2022.

[3] N. Reusch et al., "Neutrophils in COVID-19," Front. Immunol., vol. 12, p. 652470, 2021.

[4] K. Ley, C. Laudanna, M. I. Cybulsky, and S. Nourshargh, "Getting to the site of inflammation: the leukocyte adhesion cascade updated," Nat. Rev. Immunol., vol. 7, no. 9, pp. 678–689, 2007.

[5] Y. Chen et al., "Melatonin induces anti-inflammatory effects to play a protective role via endoplasmic reticulum stress in acute pancreatitis," Cell. Physiol. Biochem., vol. 40, no. 5, pp. 1094–1104, 2016.

[6] F. Mouawad, H. Tsui, and T. Takano, "Role of Rho-GTPases and their regulatory proteins in glomerular podocyte function," Can. J. Physiol. Pharmacol., vol. 91, no. 10, pp. 773–782, 2013.

[7] R. Hwaiz et al., "Rac1 signaling regulates sepsis-induced pathologic inflammation in the lung via attenuation of Mac-1 expression and CXC chemokine formation," J. Surg. Res., vol. 183, no. 2, pp. 798–807, 2013.

[8] L. A. Martinez, "Effects of Regulating Rac1 in a Mouse Model of Fragile X Syndrome," 2016.

[9] Y. Hong et al., "Effects of castanospermine on inflammatory response in a rat model of experimental severe acute pancreatitis," Arch. Med. Res., vol. 47, no. 6, pp. 436–445, 2016.

[10] O. Z. Ismail and V. Bhayana, "Lipase or amylase for the diagnosis of acute pancreatitis?," Clin. Biochem., vol. 50, no. 18, pp. 1275–1280, 2017.

[11] X. Wu et al., "Protective effect of tetrandrine on sodium taurocholate-induced severe acute pancreatitis," Evid. Based Complement. Alternat. Med., vol. 2015, 2015.

[12] T. Plusczyk, S. Westermann, D. Rathgeb, and G. Feifel, "Acute pancreatitis in rats: effects of sodium taurocholate, CCK-8, and Sec on pancreatic microcirculation," Am. J. Physiol.-Gastrointest. Liver Physiol., vol. 272, no. 2, pp. G310–G320, 1997.

[13] P. Xu et al., "Pioglitazone attenuates the severity of sodium taurocholate-induced severe acute pancreatitis," World J. Gastroenterol. WJG, vol. 13, no. 13, p. 1983, 2007.

[14] M. E. Soliman, M. A. Kefafy, M. A. Mansour, A. F. Ali, and W. A. I. I. Esa, "Histological study on the possible protective effect of pentoxifylline on pancreatic acini of l-arginine-induced acute pancreatitis in adult male albino rats," Menoufia Med. J., vol. 27, no. 4, p. 801, 2014.

DOI: http://doi.org/10.24086/biohs2024/paper.1359

[15] X.-X. Hong et al., "Systemic injury caused by taurocholate-induced severe acute pancreatitis in rats," Exp. Ther. Med., vol. 24, no. 1, pp. 1–12, 2022.

[16] Y. Wang, Y. Li, S. Gao, X. Yu, Y. Chen, and Y. Lin, "Tetrahedral framework nucleic acids can alleviate taurocholate-induced severe acute pancreatitis and its subsequent multiorgan injury in mice," Nano Lett., vol. 22, no. 4, pp. 1759–1768, 2022.

[17] E. Seyhun et al., "Tauroursodeoxycholic acid reduces endoplasmic reticulum stress, acinar cell damage, and systemic inflammation in acute pancreatitis," Am. J. Physiol.-Gastrointest. Liver Physiol., vol. 301, no. 5, pp. G773–G782, 2011.

[18] Z. Yang, X. Meng, and P. Xu, "Central role of neutrophil in the pathogenesis of severe acute pancreatitis," J. Cell. Mol. Med., vol. 19, no. 11, pp. 2513–2520, 2015.

[19] A. Abdulla, D. Awla, H. Thorlacius, and S. Regnér, "Role of neutrophils in the activation of trypsinogen in severe acute pancreatitis," J. Leukoc. Biol., vol. 90, no. 5, pp. 975–982, 2011.

[20] C. Yu, M. Merza, L. Luo, and H. Thorlacius, "Inhibition of Ras signalling reduces neutrophil infiltration and tissue damage in severe acute pancreatitis," Eur. J. Pharmacol., vol. 746, pp. 245–251, 2015.

[21] G. G. Schiattarella et al., "Rac1 modulates endothelial function and platelet aggregation in diabetes mellitus," J. Am. Heart Assoc., vol. 7, no. 8, p. e007322, 2018.

[22] Z. Hasan et al., "Rho-kinase signaling regulates pulmonary infiltration of neutrophils in abdominal sepsis via attenuation of CXC chemokine formation and Mac-1 expression on neutrophils," Shock, vol. 37, no. 3, pp. 282–288, 2012.

DOI: http://doi.org/10.24086/biohs2024/paper.1359