Rac-1 Signaling Regulate Tissue Damage in Sever Acute Pancreatitis

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Abstract

The mortality rate from severe acute pancreatitis remains high (20–30%) even with medical care. By giving rats a large intraperitoneal dosage of L-arginine, a novel kind of experimental necrotizing pancreatitis was created. Acinar cell necrosis that is dose-dependent and selective is produced using this non-invasive approach, which is also very repeatable. This model is not only helpful for researching the pathomechanisms of acute necrotizing pancreatitis, but it is also quite effective for monitoring and influencing changes in the disease's progression over time.

Here, our goal was to investigate the possible role of Rac-1 in acute severe pancreatitis. The rats were given intraperitoneal L-arginine, which caused pancreatitis. Animals were given normal saline in group 1 and a high dosage of L-arginine in group 2 (250 mgx2/100 gm) at intervals of one hour. Group 3 received Rac-1 NSC23766 (5 mg/kg) 15 minutes before to L-arginine administration.

Blood amylase levels were considerably lower in the Rac-1 inhibitor group (92.66 ±17.1) than in the L-arginine treatment group (216.1 ± 18.47) compared to the control group (57.38 ±7.5). The L-arginine-induced rise in myeloperoxidase serum levels was significantly reduced by Rac-1 treatment, going from (61.05± 15.02) to (12.34±3.3) in the Rac-1-treated group, whereas the control group's myeloperoxidase level was (12.04±2.56). Furthermore, when compared to the control group (1.32 ±0.11), the Rac-1 group's serum interleukin-6 levels in response to the L-arginine challenge dropped from (6.01±0.53) to (3.76±0.13) after pre-administration of Rac-1. C-reactive protein was likewise considerably lower in the Rac-1-treated group (0.838 ±0.083) and L-arginine-treated group (2.09±0.3) in this study compared to the control group (0.268 ±0.063).

The inflammation of the pancreas is followed by neutrophil infiltration, bleeding, necrosis, vaculation, and edema development. Lung tissue also experiences tissue degradation, thickening of the alveolar wall, immune cell infiltration, and lymph node thickness. Our findings show that Rac-1 inhibitors protect against chronic inflammation and lung and pancreatic tissue damage brought on by large doses of L-arginine in acute pancreatitis. Rac-1inhibitor may be helpful in treating patients with severe acute pancreatitis, according to these studies.

In conclusion, Rac-1 attenuated and regulates tissue damage through regulation of biomarkers such as amylase; myeloperoxidase and interleukin-6, also decrease neutrophil infiltration in tissues in sever acute pancreatitis.

Abbreviations: (AP) acute pancreatitis; (IL-6) interleukin-6; (i.p.) intra-peritoneal; (MPO) myeloperoxidase.

1- Introduction

Acute pancreatitis (AP) is an inflammatory condition that can occasionally affect distant organ systems as well as peri-pancreatic tissues (Guidelines 2013). Clinically, it is characterized by high blood pancreatic enzyme levels and abdominal discomfort (Nwafo 2017). The disease's severity varies widely, ranging from mild forms that solely affect the pancreas to devastating multi-systemic organ failure that can result in death (Pandol, Saluja et al. 2007). A significant portion of cases (20%) are clinically severe and linked to elevated rates of morbidity and mortality (Chatila, Bilal et al. 2019) .

Due to the discharge of pancreatic broth into the bloodstream, acute pancreatitis can cause pancreatic toxemia, which impairs pulmonary, renal, and cardio-circulatory systems (Bassi, Falconi et al. 2001).

Remarkably, about 70% of cases of acute pancreatitis are caused by gallstones and alcohol use (Wang, Gao et al. 2009). Some medications, such azathioprine and sulfonamides, are responsible for the remaining occurrences (Rawla, Bandaru et al. 2017). Certain infections, including those caused by bacteria, viruses, or parasites; trauma; hyperlipidemia; hypercalcemia; and smoking cigarettes (Edderkaoui and Thrower 2013).

Both acute and chronic pancreatitis are brought on by injuries that trigger the pancreas to start breaking down its own enzymes (Lin, Gao et al. 2015). Under normal circumstances, the pancreas is protected from self-digestion by its released enzymes via a number of processes. Rats can develop acute pancreatitis through non-invasive (hormone-, alcohol-, immune-, diet-, gene-, or L-arginine-induced) or invasive (closed duodenal loop, antegrade pancreatic duct perfusion, biliopancreatic duct injection, combination of secretory hyperstimulation with limited exposure to

intraductal bile acid, vascular-induced, ischemia/reactivity) methods (Su, Cuthbertson et al. 2006).

The first description of the L-arginine-induced acute pancreatitis model was made in rats (Mizunuma, Kawamura et al. 1984). This basic amino acid-induced pancreatitis model has grown in popularity due to its affordability, non-invasiveness, and ease of use. It requires only one or two intra-pertoneal injections to produce severe necrotizing disease without causing any morphological changes in the Langerhans islets (Kui, Balla et al. 2015). The mechanism by which L-arginine causes pancreatitis is not fully understood. Some studies suggests that nitric oxide (NO), inflammatory mediators, and oxygen-free radicals are important in the disease's development (Hegyi, Rakonczay Jr et al. 2004).

For many years, researchers have been searching for a suitable treatment for acute pancreatitis. There are currently relatively few effective remedies for the illness, and despite hundreds of clinical trials, no medication therapy has been approved (Afghani, Pandol et al. 2015). The most often requested test for acute pancreatitis (AP) diagnosis is serum amylase levels. The great majority of patients of acute pancreatitis frequently show a three-fold or greater increase in this level. However, because AP can and does occur with normal levels of amylase, its significance in diagnosis and prognosis has often been unclear and controversial (Hall, Stephenson et al. 2015).

Myeloperoxidase (MPO) levels in the serum were utilized as a marker of neutrophil infiltration. MPO levels increased in response to L-arginine challenge (Asaduzzaman, Zhang et al. 2008). Serum MPO activity caused by L-arginine was reduced by administering Rac-1. The accumulation of activated neutrophils in the lung and pancreas is a component of the systemic inflammatory response in severe

AP. In fact, the challenge with L-arginine significantly raised the serum levels of MPO. The elevated MPO activity in these organs was decreased by Rac-1 treatment.(Bhatia and Hegde 2007).

The 26 kDa glycoprotein interleukin-6 is produced by a variety of cells, including neutrophils, fibroblasts, B-cells, T-cells, monocytes, and pancreatic acinar cells (Nieminen, Maksimow et al. 2014). It has pleiotropic properties and inhibits the production of TNF and IL-1 in acute inflammation, which increases the synthesis of IL-1Ra and causes the release of the soluble TNF-receptor (Xing, Gauldie et al. 1998). The differentiation of Th17 cells, macrophages, and antibody-producing cells is stimulated by interleukin-6 (Kay, Smith et al. 2017), Additionally, it causes hepatocytes to produce C-reactive protein (BM, Krüger et al. 2005) and(Naskalski, Kusnierz-Cabala et al. 2003). Interleukin-6 also promotes the release of IL-10, which functions as an anti-inflammatory mediator (Kay, Smith et al. 2017). Serum IL-6 levels rise prior to severe pancreatic edema and necrosis in rats with experimental AP (Czakó, Takács et al. 2000). In humans, IL-6 is a more accurate indicator of the severity of AP than C-reactive protein, which is the most often used indicator in clinical practice (Kay, Smith et al. 2017). Interestingly, a new study (Jain, Midha et al. 2018) found that measuring serum IL-6 improves SIRS's ability to detect severe AP.

Making the early distinction between moderate and severe pancreatitis is one of the main clinical issues (Leser, Gross et al. 1991). As a result, a number of prognostic indicators have been put out to identify patients who are at high risk. Increased serum concentrations of acute-phase proteins, especially C-reactive protein (CRP), have recently been highlighted for their prognostic significance (Puolakkainen, Valtonen et al. 1987).

Numerous studies have indicated that neutrophils may be involved in severe AP, and their infiltration is a crucial aspect of the inflammatory response (Yu, Merza et al. 2015). A number of sequential processes, such as P selectin and Lymphocyte Antigen-1, facilitate neutrophil invasion (Hartman, Abdulla et al. 2012) . A member of the Rho family, Rac-1 is a tiny GTP-binding protein that controls a number of biological processes, including the activation of NADPH oxidase, a key intracellular source of reactive oxygen species (ROS) (Choi, Sicklick et al. 2006).

The purpose of this study was to investigate the regulatory role of Rac-1 in the levels of amylase, MPO, IL-6, CRP, neutrophil infiltration, and lung and pancreatic tissue damage in acute pancreatitis. Using these experimental models of severe acute pancreatitis in rats, we administered two doses of (500 mg/100 g body weight) of L-arginine intraperitoneally, each containing 250 mg, at one-hour intervals. Moreover, 15 minutes before the initial L-arginine injection dose (pre-treatment), 5 mg/kg body weight of Rac-1 was administered intraperitoneally.

2. Methodology

2.1. Animals and housing

Male rats *Rattus norvegicus* were bred in the animal house of Biology Department - Education College -Salahaddin University-Erbil. During the entire period of experiments the rats were kept in special cages with a steel stainless wire mesh top to hold fed with standard rodent diet and water *ad libitum*. The room temperature was kept at about 22±4 ^{o}C and the light dark cycle was kept in about 12/12 hours.

2.2. Preparation of L-Arginine dose

The L-Arginine from sigma Aldrich, 500 mg/100g body weight (B.W) was prepared by dissolving it in distal water, each rat given specific volume according to exact B.W intra-pertonially (i.p.)

2.3. Rac-1 inhibitor

Rac1 inhibitor, NSC 23766 from Tocris (Bristol, United Kingdom) is a member of the Rho family, a small GTPase that functions as a molecular switch, playing a role in the regulation of various essential cellular functions (Mouawad, Tsui et al. 2013). The signal transducer, Rac-1 powder, dissolved in distill water Rac-1 is widely expressed and regulates a number of processes linked to inflammatory reactions, such as chemotaxis, cell adhesion, and vascular permeability (Hwaiz, Hasan et al. 2013).

2.4. ELISA

Amylase and myeloperoxidase levels in the serum as well as IL-6 levels in the serum were analyzed by use of double-antibody Quantitative enzyme linked immunosorbent assay kits (ELISA) (ELK Biotechnology from USA). While C-reactive protein was determined by fully automated biochemistry analyzer from GenoTEK model (smart- 150) china.

3. Design of the experiments

Twenty-four adult male rats weighting 240-300 gm and 12 weeks of age were divided equally into 3 groups each group eight rats were injected i.p. as the following:

Group 1 (control): Rats were given standard diet and tap water and injected with D.W i.p. twice one hour interval according to B.W.

Group 2 (L-arginine alone): Rats were given standard diet and tap water and L-arginine two dose of 250mg/100gm B.W one hour interval i.p.

Group 3:- Rac-1 +L-Arginine (pre-treatment): Rats were given standard diet and tap water and Rac-1inhibitor 5mg/kg B.W 15 minutes prior to L-arginine two dose of 250mg/100gm B.W 1 hour interval i.p.

-Induction of Pancreatitis in Rats

L-arginine hydrochloride was dissolved in normal saline to create a sterile solution. Non-fasting rats received an intraperitoneal injection of the L-arginine solution at a ratio of 250 mg/100 g (Dawra, Sharif et al. 2007). Animals were returned to the cages and allowed free access to feed and water. After one hour, animals were administered a second dose of L-arginine (250mg/100g) in saline. Control group was received saline alone. While the third group of rats received the same dose of L-arginine (250mg/100gm B.W.), after 15 minutes of Rac-1 injection (5mg/kg B.W.) intera-pertoneally. Animals were sacrificed after 72hours and a blood sample taken from the heart and tissue collected (pancreas and lung). Tissue samples for histology were fixed in 10% formalin.

-Anesthesia

The rats were anaesthetized by a combination of Ketamine (Rotexmedica and Tritta Germany) and xylazine (Xyla Ject Holland). Ketamine and xylazine were injected intraperitoneally in a dose of 90 mg/kg B.W and 10 mg/kg B.W, respectively.

-Histological sectioning

A part of pancreas and lung tissues were preserved in fixative solution (10% formalin) exposed to serial processes begin with dehydration, clearing and impregnation using a series of graded ethanol in ascending concentrations then immersed in xylene. Finally embedded in paraffin wax and cooled. Paraffin sections were cut by rotary microtome, and then stained with haematoxylin and eosin (Layton, Bancroft et al. 2019).

-Statistical analysis

All data were expressed as Mean ± S.D, data analysis done by GraphPad Prism 10, comparison was made using one-way ANOVA, results compared by ANOVA and Duncan to determine significance among groups. Values were considered to be significantly different when $P<0.05$.

5. Results

Rac-1 controls amylase pancreatic enzyme

Amylase activity was identified as a tissue damage biomarker in pancreatitis to evaluate the involvement of Rac-1 in severe AP. We found that the rat's intraperitoneal (i.p.) injection of L-arginine significantly raised the serum amylase concentration by around four folds (Table 1). Treatment with the Rac-1 inhibitor (5 mg/kg) 15 minutes before L-arginine injection reduced the retrograde injection of L-arginine-induced serum amylase activity levels from 216.1 ± 18.47 ng/ml to 92.66 ± 17.1 ng/ml, while the control group's amylase concentration was 57.38 ± 7.5.

Myeloperoxidase Activity in Response to L-Arginine

When acute pancreatitis develops, neutrophil sequestration occurs in the pancreas. One biochemical indicator of neutrophil infiltration has been the

Groups / parameter	Control	L-Arginine alone	Rac-1+L-Arginine
S. Amylase (ng/ml)	**57.38 ±7.5[a]**	**216.1 ± 18.47[c]**	**92.66 ±17.1[b]**
S. MPO (ng/ml)	**12.04 ±2.56[a]**	**61.05± 15.02[b]**	**12.34 ±3.3[a]**

measurement of myeloperoxidase (MPO) activity in serum. Rats given L-arginine had a markedly elevated serum level of MPO activity in contrast to the saline-only control group. According to table 1, we discovered that L-arginine considerably raised serum MPO levels by five times. 15 minutes before to the injection of L-arginine, pretreatment with Rac-1 (5 mg/kg) significantly reduced serum MPO levels in the L-arginine group and non-significantly in the control group.

The MPO level in the L-arginine alone group was 61.05±15.02 ng/ml, whereas the MPO level in the Rac-1 + L-arginine group was 12.34±3.3 ng/ml. This means that the Rac-1 treatment significantly lowers the MPO level in rats with pancreatitis, while the MPO level in the control group was 12.04±2.56 ng/ml.

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Table (1): Effect of L-Arginine and Rac-1 inhibitor on serum Amylase and Myeloperoxidase in male rats.

Effect of L-arginine on interlukin-6

Groups / parameter	Control	L-Arginine alone	Rac-1+L-Arginine
IL-6(pg/ml)	**1.32 ±0.11[a]**	**6.01± 0.53[c]**	**3.76 ±0.13[b]**

Rats given L-arginine intraperitoneally (i.p.) had considerably higher serum levels of IL-6 than the control and Rac-1 groups, as indicated in Table 2. Rats in group three had lower serum levels of IL-6 after pretreatment with Rac-1 than those in the L-arginine group. Serum levels of IL-6 were 1.32±0.11 pg/ml in the control group and dramatically increased to 6.01±0.53 pg/ml in the L-arginine group. However, following treatment with Rac-1in group three (Rac-1+L-Arginine), they significantly dropped to 3.76 ±0.13 pg/ml.

Effect of L-arginine on CRP level

Treatment with Rac-1 reduced the non-significant increase in CRP serum levels caused by L-arginine injection. Table 2 reported the effect of Rac-1 on CRP concentration, showing a non-significant decrease in the (Rac-1+L-arginine) group (0.838±0.083) compared to group two (L-arginine alone) 2.09±0.3, while the Rac-1 group showed a non-significant increase (0.268±0.063) compared to the control group.

CRP(mg/dl)	**0.268 ±0.063[a]**	**2.09 ±0.3[a]**	**0.838 ±0.083[a]**

Table (2): Effect of L-Arginine and Rac-1 inhibitor on serum IL-6 and CRP in male rats.

Histopathological results: H&E

1- Pancreas tissue

The tissue of the control pancreas had typical pancreatic anatomy, with lobules divided by thin interlobular septa. The acini, ducts, and islets of Langerhans make up the pancreatic lobules. Figure 1a and b showed well-formed ducts bordered by cuboidal epithelium. Significant fluid collection, cell swelling, and alteration of histoarchitecture were observed in the L-arginine-administered group (Fig. 1 C). In response to an infusion of L-arginine, acinar cell vacuolization and cytoplasmic expansion were observed in pancreatic tissue (Fig. 1d).

Furthermore, as seen in (Fig.1e), the injection of high dosages of L-arginine results in the broad necrosis of acinar cells. After rats were injected with 500 mg/100g body weight of L-arginine, (fig. 1-f) demonstrated the infiltration of many immune cells in response to tissue inflammation, whereas Langerhans cells remained unaffected. Finally, when Rac-1 was injected 15 minutes before to the injection of L-arginine, (fig. 1g) displayed minimal immune cell infiltration and minimal cell degradation.

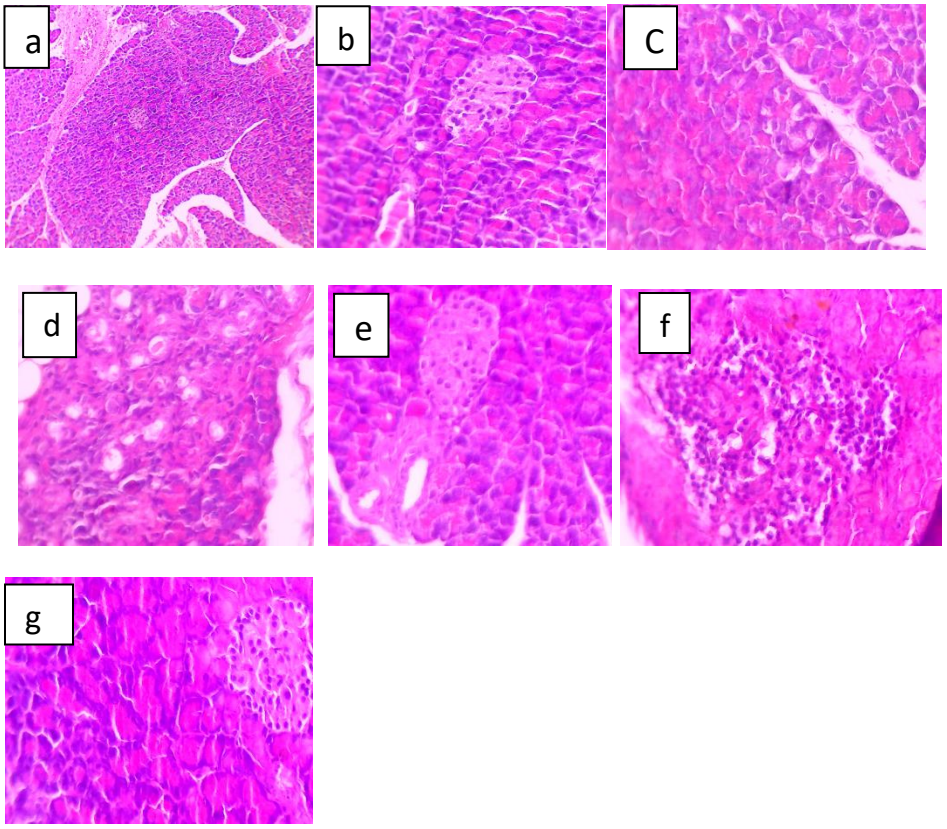

Figure1:- -a: normal section of pancreas 100x show normal pancreatic structure, appeared as lobules separated by thin interlobular septa. The pancreatic lobules composed of acini, ducts and islets of Langerhans. b: normal section of pancreas 400x , show well-formed ducts lined with cuboidal epithelium were observed c- pancreatic cells 100x shows swelling of pancreatic acini cells d- section of pancreatic cells 400x show formation of vacule (vaculization) in response to L-arginine injection I.P. and expand of cytoplasm inside the cells. e-Pancreatic cell 400x show necrosis of acini cells due to administration of high dose of L-arginine I.P without langerhanse cells affecting.. f- section of pancrease cell show infilitration of immune cells. g- pancrease tissue of Rac-1 group showed little cell infiltration and little degeneration in comparision with L-arginine treated group.

2- Lung tissue

Acute pancreatitis (AP) is an inflammatory disease that can range from a mild form to a severe necrotic condition that may result in acute lung injury. Figure 2-a shows normal lung tissue with normal alveolar sac and normal alveolar wall. We discovered that intra-peritoneal injection of a high dose of L-arginine (500 mg/100 gm B.W.) causes lung damage as evidenced by histological change (Figure 2-b), in which the alveolar wall thickens, indicating that the alveolar wall becomes multilayered of cells. Additionally, we found that the injection of L-arginine (500 mg/100 gm B.W.) results in immune cell infiltration, as shown in (Fig2-c). Finally, we found another abnormality in lung tissue, which is the thickness of lymph nodules, which is shown in (Fig2-d).

In contrast to the L-arginine group, the Rac-1-treated group exhibited minimal tissue alteration and cell infiltration. This suggests that Rac-1 has an inhibitory role in acute pancreatitis, reducing lung tissue damage and blocking the effects of L-arginine (fig.2-e).

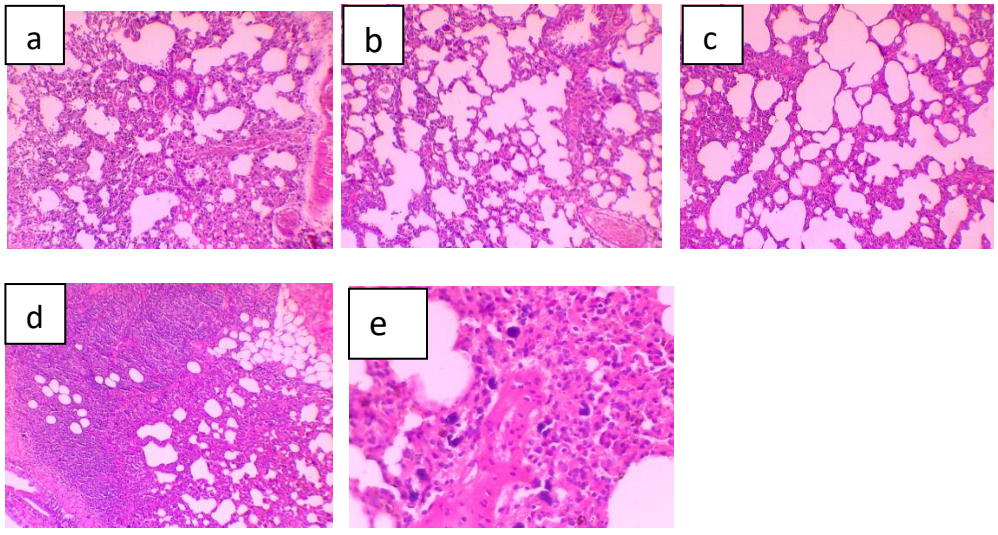

Figure 2:- a-Normal luge tissue show normal alveolar sac and normal alveolar wall 100x. b- section of lung show thickness in the alveolar wall due to injection of L-arginine 100x. c- Infiltration of immune cell especially neutrophils, in the rats treated with L-arginine 400x. d- 100x lung tissue of L-arginine group show thickness in lymph node. e- lung tissue show little cell damage and little infiltration due to Rac-1 treatment.

DISCUSSION

A novel and highly repeatable rat model of acute pancreatitis was created and described in this article by administering a high dosage of L-arginine. intraperitoneal injection of 500 mg/100 g twice, with a one-hour gap between each dosage of 250 mg/100 g B.W (Mizunuma, Kawamura et al. 1984).

Since rats are known to develop acute pancreatitis when given large doses of L-arginine, a small number of labs have utilized this as a model to research pancreatitis. The exact mechanism of L-arginine-induced pancreatitis is still unknown, and it has not been completely described. Therefore, in this investigation, we demonstrated that administering L-arginine intraperitoneally causes pancreatitis, which is consistent with (Tani, Itoh et al. 1990).

Pancreatitis was caused by administering two doses of L-arginine (500 mg/100 g B.W.) one hour apart. The pancreatitis-related alterations began to show early, progressed progressively, and peaked at 72 hours. Consequently, 72 hours was selected as the time point for examining tissue alterations (Hardman, Shields et al. 2005).

The pancreas is primarily responsible for secreting the digesting enzyme amylase. In cases of acute pancreatitis, amylase levels can rise rapidly, peaking three to six hours after symptoms start and remaining elevated for up to five days (Ismail and Bhayana 2017). Previous studies have shown the critical role that amylase plays in the development of acute pancreatitis. In contrast to the control group (57.38±7.5ng/ml), we observed a notable increase in serum amylase levels (216.1±18.47ng/ml) following the retrograde intraperitoneal injection of L-arginine in our current investigation (Table 1). Previous research has also demonstrated a rise in amylase levels after severe acute pancreatitis, which is consistent with our findings, an increase in serum amylase return to tissue damage and cell necrosis of

acinar cell in pancreas (Wu, Li et al. 2015) and (Xu, Zhou et al. 2007) which also approved by our study of pancreas tissue.

However, our research showed that serum amylase was significantly reduced after pretreatment with the Rac-1 inhibitor (5 mg/kg) (Table 1). This emphasizes how acute pancreatitis involves both amylase and Rac1. Additionally, our research aligned with the findings of (Dawra, Sharif et al. 2007) They showed that giving rats L-arginine dramatically raised their blood amylase levels in comparison to the control group. This is a marker of damage to the pancreatic acinar cells and tissue damage in the pancreas. Additionally, this investigation supported (Chang and Chung 2011) research who observed that rats given a high dosage of L-arginine had higher serum amylase.

Histological analysis revealed considerable pathological alterations in the pancreatic tissue of rats given L-arginine (figure 1b,c,d,e), which are suggestive of serious tissue injury. The amount of tissue damage and immune cell infiltration in our investigation was significantly reduced by the injection of the Rac-1inhibitor (5 mg/kg). Prior research has also demonstrated that severe acute pancreatitis causes damage to the pancreatic tissue. (Dawra, Sharif et al. 2007). Our results indicated that the Rac-1 inhibitor reversed alterations in immune cell infiltration in both pancreas and lung. (Yang, Meng et al. 2015) mentioned the importance of neutrophil infiltration in severe acute pancreatitis. This pattern is consistent with our findings, as seen in the pancreatic section (Figure 1).

In this context, MPO levels act as an indicator of neutrophil infiltration, we noted a significant increase of serum MPO level after administration of L-arginine (500mg/100gm) body weight intera-peritoneally (Table 1). Interestingly, Pre-administration of the Rac-1 inhibitor markedly decreased L-arginine-induced MPO

levels in the serum. These findings align with those reported in earlier studies(Dawra, Sharif et al. 2007).

Recent study reported that IL-6 seems to be an important link between local inflammation in the pancreas on one hand and systemic inflammation and lung damage on the other hand (Merza, Rahman et al. 2014). We therefore examined serum levels of IL-6 in pancreatitis animals and found that L-arginine markedly increased the circulating levels of IL-6 (**6.01± 0.53)** as showed in table 2. Notably, serum levels of IL-6 were greatly reduced in Rac-1 treated animals by 50% when compared with L-arginine group (**3.76±0.13)** that shown in table 2. While IL-6 level in the control group was (**1.32 ±0.11**).Whether this Rac-1 inhibitor regulated formation of IL-6 plays a mechanistic role in pancreatitis-associated lung damage and mortality remains to be studied in the future. Also this study is supported by the (Fisic, Poropat et al. 2013) who showed that IL-6 level in acute pancreatitis increase significantly with the control group, moreover, we discovered that the Rac1 NSC23766 inhibitor strongly decreased IL6 levels in rats induced with AP, this result clarify in table 2. Rac1 inhibitor may thus be a therapeutic agent for controlling AP by inhibiting chemokine, a signaling molecule in exaggerated inflammation. This study was also in agreement with(Rao and Kunte 2017) who demonstrated that interleukin-6 increased in acute pancreatitis and its early marker for severity of acute pancreatitis.

Although a number of laboratory and clinical studies have shown that interleukin-6 is the principal mediator of the acute phase protein response (Heath, Cruickshank et al. 1993), we found in this study the significant increase in CRP (**2.09 ±0.3)** in acute pancreatitis after administration of L-arginine to rats (500mg/100gm) intra-pertoneally as showed in table2. There is evidence, mainly

from experimental studies, that the cytokine interleukin- 6 induces the hepatic synthesis of CRP and other acute-phase proteins (Andus, Geiger et al. 1987), in this study we also show an increase in C-reactive protein parallel to increase in IL-6, so according to previous study there is a strong relation between CRP and interleukin-6; moreover, we discovered that the Rac-1 inhibitor strongly decreased CRP levels **(0.838 ±0.083)** in rats induced with acute pancreatitis, still CRP is not effective marker for severity of acute pancreatitis as demonstrated by (Fisic, Poropat et al. 2013) , CRP levels on admission did not reliably differentiate between severe and mild cases of acute pancreatitis, but reached statistical significance when measured on the 3rd day after infection. This could be one of the reasons why CRP was not found to be an effective marker for severe pancreatitis.

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.

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