

Comparing the therapeutic action of octreotide, trimetazidine and their combination by pro-inflammatory and enzymatic changes in L-Arginine induced acute pancreatitis in male rats

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Abstract: Acute pancreatitis continues to be associated with significant rates of mortality and morbidity, and therapeutic options are still very limited. Various theories have been suggested regarding the pathophysiology of acute pancreatitis, lot of research into different medical treatments for the treatment of acute pancreatitis, but it is not clear what benefits each treatment has, or indeed if any medical treatment is beneficial apart from supportive treatment. To clarify the potential therapeutic effect of octreotide, trimetazidine, and their combination in acute pancreatitis. Acute pancreatitis was induced by L-arginine and treated with octreotide subcutaneously, trimetazidine intraperitoneally and combination therapy by octreotide and trimetazidine. The rats were followed for 24 hours. At the 24th hour we determined serum levels of TNF- α , IL-1 β , lipase, and amylase. TNF- α ($p < 0.001$), IL-1 β ($p < 0.001$), lipase ($p < 0.001$), and amylase ($p < 0.001$) serum levels were significantly lower in combination group as compared with both octreotide and trimetazidine groups. Combination treatment markedly decreases biochemical and enzymatic changes in acute pancreatitis, thus ameliorate pancreatic injury in L-arginine induced acute pancreatitis.

Keywords: acute, pancreatitis, arginine, octreotide, trimetazidine.

INTRODUCTION

Acute pancreatitis (AP) is defined as an acute inflammatory process of the pancreas that usually associated with a severe pain in the upper abdomen; in most instances, blood levels of pancreatic enzymes, including amylase and lipase, are increased to at least three times the upper limit of normal.

The inflammatory reaction in AP results in edema of the pancreas and extensive local and systemic effects. AP may occur as a new event or as a recurrent condition. In about 85% of cases, the disease takes a mild course with complete disappearance of clinical symptoms in a few days. In a small group of about 15-20% of patients, the disease takes a severe course, necrotising pancreatitis. This may culminate in multi-organ failure and death in about 15% - 40% of these patients [1].

AP continues to be associated with significant rates of mortality and morbidity, and therapeutic options are still very limited. Various theories have been suggested regarding the pathophysiology of acute pancreatitis, lot of research into different medical treatments for the treatment of acute pancreatitis, but it is not clear what benefits each treatment has, or indeed if any medical treatment is beneficial apart from supportive treatment [2].

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

A total of 45 sprague dawley adult male rats weighing 200-300g, were purchased from Animal Resource Center, veterinary medicine college, Al-Qassim University, the National Center for Drug Control and Researches, Iraq. The animals were look healthy and the animals were lived in the animal house of College of pharmacy / University of Kufa in a temperature ranged ($25 \pm 2^\circ\text{C}$) room, with vacillating 12-h light/12-h dark cycles and the animals were permitted for free access to water and chow diet until the beginning of the study.

Study design

Rats were randomized into five groups ($n=9$) in each group as following: Control group: injected with normal saline with 2 doses of 1.25 ml/100 g separated by 1 hr interval, and injected with normal saline in an equivalent volume to trimetazidine and octreotide solutions. Induction group: injected with L-arginine with 2 doses of 250 mg/100gm separated by 1 hr

interval, and injected with normal saline in an equivalent volume to trimetazidine and octreotide. Octreotide treated group: injected with single octreotide dose of 4 µg/kg sc after induction with L-arginine. Trimetazidine treated group: injected with single trimetazidine dose of 10 mg/kg ip after induction with L-arginine. And Combination group: injected with single trimetazidine dose of 10 mg/kg ip and single octreotide dose of 4 µg/kg sc after induction with L-arginine.

Induction of acute pancreatitis

After weighing the animal accurately, we calculate the volume of L-arginine hydrochloride solution (20%) to be injected using a dose of 250 mg/100 g body weight (1.25 ml/100 g).

Then filling the solution in a sterile 2 ml syringe with 25 G 5/8” needle and inject i.p. and Putting the animal in a clean cage with feed and water. We do the injection in a laminar air flow hood with filters. Waiting for 1h and then deliver the 2nd dose i.p. and return the animal to its cage. Rats become slow after the 2nd injection but gradually recover [3].

ELIZA

Twenty four hours following the induction of acute pancreatitis, the blood was drawn using direct needle puncture of the heart. Samples of the blood were left to clot at 37°C and centrifuged at 3000 rpm for 10 min; Serum was pulled, and analyzed for detection of serum lipase, amylase, IL-1β, TNF-α.

RESULTS

At the end of study, it is found that Serum TNF-α, IL-1β, IL-6, lipase, and amylase serum levels were significantly elevated in induction group as compared with that of control group. Both octreotide and trimetazidine significantly counteract the increase in serum levels of TNF-α, IL-1B, lipase, and amylase.

While combination therapy of octreotide plus trimetazidine decreases these parameters significantly as compared with induction group and even with octreotide or trimetazidine groups.

Results are shown in figures 1,2,3 and 4.

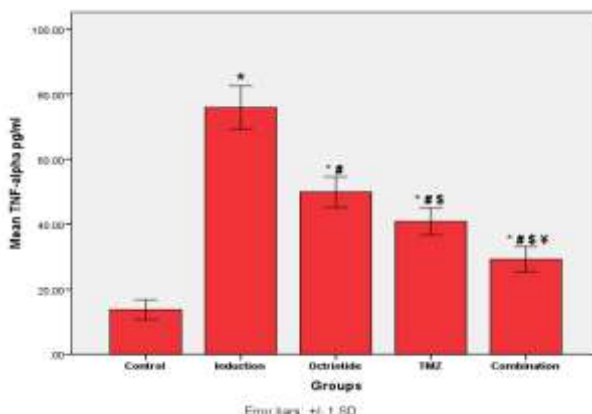


Fig-1: The mean level of TNF-α (pg/ml) in the five experimental groups

*significant vs control group #significant vs induction group \$significant vs octreotide ¥significant vs TMZ. Data are expressed as mean ± SD

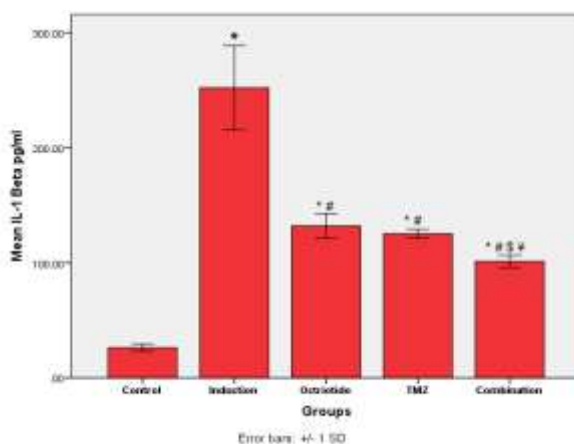


Fig-1: The mean level of IL-1β (pg/ml) in the five experimental groups

*significant vs control group #significant vs induction group \$significant vs octreotide ¥significant vs TMZ. Data are expressed as mean ± SD

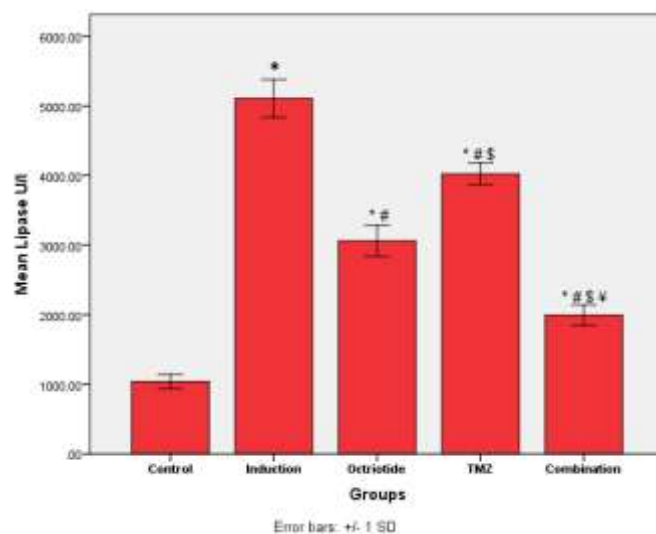


Fig-3: The mean level of lipase (U/l) in the five experimental groups

*significant vs control group #significant vs induction group §significant vs octriotide ¥significant vs TMZ. Data are expressed as mean ± SD

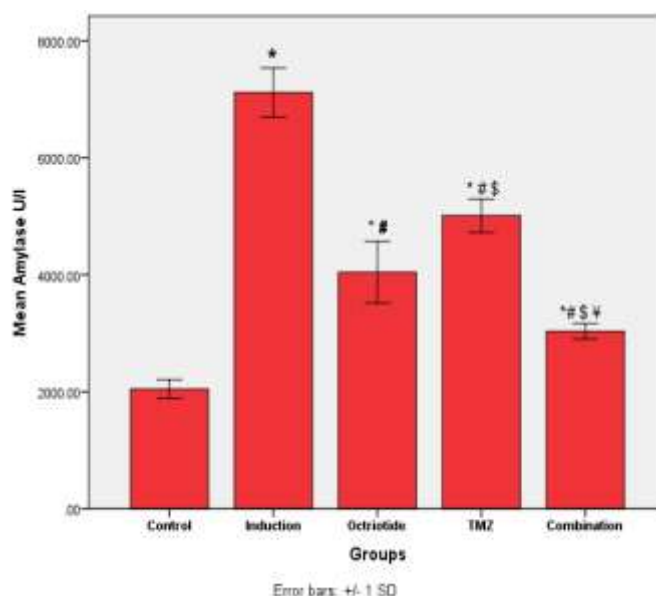


Fig-4: The mean level of amylase (U/l) in the five experimental groups

*significant vs control group #significant vs induction group §significant vs octriotide ¥significant vs TMZ. Data are expressed as mean ± SD

DISCUSSION

Acute pancreatitis is an inflammatory condition of the pancreas, with varying involvement of other tissues and organs. The disease includes a spectrum of pancreatic lesions, varying from parenchymatous edema to severe hemorrhagic pancreatitis, with necrosis, infection and organ destruction [4].

Acute pancreatitis is a disease with high morbidity and mortality. Various theories have been suggested regarding the pathophysiology of acute pancreatitis, yet the underlying mechanism is not clearly understood. Oxygen free radicals (OFRs) and

basic pro inflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6), which play a role in acute pancreatitis and other systemic inflammatory conditions, have been suggested to be responsible for the local tissue damage and multiple organ failure that occur during acute pancreatitis. The OFRs lead to membrane lipid peroxidation, changes in the main components of the cytoplasm and early activation of digestive enzymes of pancreatitis, and they initiate protein damage in acute pancreatitis [5].

Effect of acute pancreatitis on study parameters after L-Arg. Induction:

The present study shows significant elevation ($P < 0.001$) in serum level of pro-inflammatory cytokines (TNF- α and IL-1 β) in induction group as compared with the control group. Similar results obtained by [6] WANG, Y *et al.* mentioning that in AP, TNF- α and IL-1 β may play an important role in stimulating the inflammatory response in the animal experimental studies, the levels of IL-1 β and TNF- α increased significantly in the blood and in pancreatic tissues, and associated with the severity of the disease.

Also Yilmaz, E. E *et al.* [7] showed that there is a significant increase in IL-1 β and TNF- α level in AP induction group.

The oxidative stress is intensely involved in the inflammatory processes of AP since the OFRs are the major pro oxidant agents and in the same time acts as inflammatory mediators through the stimulation of leukocytes activation, adhesion, and then emigration in addition to improving the expression of other inflammatory mediators, such as cellular adhesive molecules [8].

Also the present study shows significant increase ($P < 0.001$) in serum levels of the lipase and amylase in induction group as compared with the control group. Similar results were obtained by Nader, M. A *et al.* [9] and Aziz, N.M *et al.* [10] and they confirmed that there is a significant increase was detected in serum levels of lipase and amylase in L-Arg treated rats compared to the control group.

Effect of treatment on study parameters after L-Arg. Induction:

Effect of treatment on proinflammatory cytokines (TNF- α and IL-1 β) after L-Arg. Induction:

The present study shows that octreotide treatment significantly decrease the serum levels of pro-inflammatory (TNF- α and IL-1 β) cytokines as compared with the induction group, these findings also obtained by [11] who study the effects of octreotide on hepatic ischemic reperfusion injuries in a rabbit models and shows that the level of both TNF- α and IL-1 β are lower significantly in octreotide group than the disease group. Zhang, XP *et al.* [1] & Tian, H. *et al.* [13] also both proved that octreotide can significantly decrease serum TNF- α in acute pancreatitis.

TMZ treatment significantly decrease the serum level of pro-inflammatory (TNF- α and IL-1 β) cytokines as compared with the induction group, these findings also obtained by Tanoglu *et al.*, [14] who confirmed that TMZ can significantly decrease serum TNF- α and IL-1 β in the treatment of experimental sepsis in rat model.

While Tanoglu *et al.*, [15] found that Serum IL-1 β levels was significantly lower during TMZ treatment and there was no significant differences in serum TNF- α level, contrary Vinokurov *et al.*, [16] show that TMZ can Inhibits the Secretion of TNF- α and may reduce the inflammatory response as a result of the blockade of secretion of pro inflammatory cytokines, which may occur due to a reduced expression of the components of the receptor for endotoxin, and that is confirmed by our study that shows a significant decrease in TNF- α level in TMZ group as compared with both induction group and octreotide group.

Combination treatment significantly decrease the serum level of pro-inflammatory (TNF- α and IL-1 β) cytokines as compared with the induction group.

Also there is a significant decrease in serum level of pro-inflammatory cytokines in combination therapy as compared with the single therapy model with either octreotide or trimetazidine alone.

According to our knowledge, there are no studies on using combination therapy with octreotide and trimetazidine in acute pancreatitis in a rat model, and this study is the first.

Effect of treatment on pancreatic enzymes (lipase and amylase):

The present study shows that octreotide treatment significantly decrease the serum level of pancreatic enzymes (amylase and lipase) as compared with the induction group, these findings also obtained by Chen *et al.*, [17] that showed a significant decrease in lipase and amylase serum levels after octreotide treatment in experimentally induced pancreatitis in adult rats. Woeste *et al.*, [18] also showed a significant decrease in lipase level after octreotide treatment. Additionally Kafali *et al.*, [19] proved that octreotide can significantly lower amylase level in rats in early dose immediately after AP induction. Zhou *et al.*, [20] proved that octreotide can decrease serum amylase level in canine model AP.

Our study shows a significant reduction in lipase and amylase levels in octreotide group as compared with TMZ group proving the strong anti-secretory activity of octreotide on the pancreas.

TMZ treatment significantly decrease the serum level of the pancreatic enzymes (amylase and lipase) as compared with the induction group, these findings also obtained by Tanoglu *et al.*, [15] showing that TMZ can significantly reduce serum lipase and amylase levels in rat after induction of AP. Yenicieroglu *et al.*, [5] also showed that TMZ significantly decreased amylase levels in L-Arg induced rats.

Combination treatment significantly decreases the serum levels of lipase and amylase as compared with the induction group.

Also there is a significant decrease in serum levels of lipase and amylase in combination therapy as compared with the single therapy model with either octreotide or trimetazidine alone.

According to our knowledge, there are no studies on using combination therapy with octreotide and trimetazidine in acute pancreatitis in a rat model, and this study is the first.

CONCLUSION

As indicated by the consequences and results of the present study, we concluded that:

- This study additionally supports the role of proinflammatory cytokine (TNF- α , and IL-1 β), and oxidative stress in the pathophysiology of L-Arg induced acute pancreatitis.
- Octreotide and trimetazidine reduce inflammatory reaction associated with L-Arg induced acute pancreatitis as evidenced by reducing pro inflammatory cytokines (TNF- α , and IL-1 β).
- Octreotide has effect on pancreatic enzymes more than TMZ, while TMZ has more anti-inflammatory and anti-oxidant abilities than octreotide as shown by the results.
- However, the effects of combination therapy (octreotide plus TMZ) were superior to octreotide or TMZ alone, The combination therapy concentrated on numerous pathological pathways of AP, like the extreme activated pancreatic enzymes, increased activity of inflammatory mechanism, and the microcirculatory dysfunction mechanism, and Therefore, the effect of combination treatment is much better than monotherapy. Furthermore, it was established that TMZ can enhance the therapeutical effect of octreotide in AP in rat. Thus, the treatment of TMZ plus octreotide can be applied as a treatment of AP more comprehensively and more efficiently.

Statistical analysis

Statistical analysis was completed by using statistical package for social sciences (SPSS) version 20 in which we used median, mean with standard deviation as descriptive statistics. Analysis of variance (ANOVA) with LSD and Mann-Whitney test for comparison between groups. P value ≤ 0.05 regarded significant.

Conflict of interest

There is no conflict of interest regarding the publication of this paper.

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