

Ibudilast Ameliorates Acute Pancreatitis through Downregulation of Interleukin-1 Beta and Lipase Enzyme

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Abstract

Background: Acute pancreatitis (AP) is severe inflammation of the pancreas that can be of two major types: mild AP (MAP) and severe AP (SAP). **Objective:** To study the therapeutic effect of Ibudilast in comparison with older drug, octreotide, in the rat model of AP. **Methods:** A total of 48 male rats were divided into 8 groups with each group consisting of 6 rats. Acute Pancreatitis was induced by L-arginine model which has a high reproducibility. Octreotide and Ibudilast were administered individually and in combination at 0, 8 and 16 hours after induction. After 24 hours of treatment, each rat was weighed and blood samples were withdrawn for ELISA test for interleukin- 1 beta (IL-1 β) and biochemical test for serum lipase, and then the pancreas was extracted for histopathological examination. **Results:** In Octreotide and Ibudilast groups there was a statistically significant decrease in serum IL-1 β , lipase enzyme, and decrease in histopathological changes. **Conclusion:** Ibudilast and octreotide can significantly attenuate the local and systemic effect of AP. Ibudilast and the combination decreased serum lipase more significantly than octreotide. Thus these drugs can be used effectively in cases of pancreatitis as it leads to high morbidity and mortality.

Key words: Acute pancreatitis, ibudilast, interleukin-1 beta, octreotide, serum lipase

INTRODUCTION

Acute pancreatitis (AP) is a severe inflammation of the pancreas, most patients with AP are diagnosed with a mild AP with mortality rate of <1% and are usually self-limiting. Severe AP accounts for 10–20% of the AP cases with mortality rate of 10–30%.^[1] During AP, the acinar cells of the pancreas produce pro-inflammatory cytokines. Neutrophils and macrophages are recruited to furnish more cytokines and reactive oxygen species (ROS).^[2] Toll-like receptor 4 participates in the pathogenesis of AP as this receptor regulates the innate and ensuing immunity, activates nuclear factor kappa B (NF- κ B), and amplifies the apoptosis.^[3] Ibudilast is a non-selective cyclic adenosine monophosphate phosphodiesterase (PDE) inhibitor. It inhibits the formation of the nitric oxide and reduces ROS. As anti-toll-like receptor 4 (aTLR4) antagonist, it has anti-inflammatory and pain modulating effects.^[4] Inhibiting phosphor stimulates the anti-inflammatory cytokines and

prevents NF- κ B by inhibiting tyrosine kinase.^[5] Thus, it has bronchodilator, vasodilator, and neuroprotective properties. Octreotide is a long-acting analog of somatostatin which inhibits exocrine secretions of pancreas; it prevents the release of many hormones including insulin, glucagon, amylase, tripsin, lipase enzyme, thyroid-stimulating hormone, and growth hormone.^[6] These hormones play a key role in pathogenesis of pancreatitis; hence, octreotide proves to be beneficial in pancreatitis. It also improves severe AP by reducing leukocyte infiltration and pancreatic tissue necrosis and can alter the metabolism of ROS.^[7] By discerning the multiple varied effects of ibudilast, especially focusing on anti-inflammatory and anti-secretory effects, we hypothesized that it could be beneficial in animal models of AP. Thus, the

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aim of this study was to demonstrate the therapeutic effect of ibudilast in comparison with an old drug, octreotide, in the rat model of AP.

MATERIALS AND METHODS

Experimental animal

The study was performed according to the America's guidelines of animal experimentation. A total of 48 adult male Sprague Dawley rats weighing 250–300 g were obtained from a certified animal house. They were placed in a 12-h light/12-h dark cycles, at a temperature of $22 \pm 2^\circ\text{C}$ and humidity of 60%–65% and were fed standard pellet diet *ad libitum*. Ethical permission was obtained from the Institutional Ethics Committee/Kufa University, Iraq/letter number: 1001–2018.

Study design

Forty-eight rats were divided into eight groups (A through H, six rats/individual group). Group A is control group, where animals were not subjected to intervention. Group B is sham group, where rats received 1.25 mL normal saline/100 g (body weight) intraperitoneally (IP). Group C is AP-induced rats received two doses of L-arginine at 250 mg/100 g (body weight) IP with 1 h gap between injections. Group D is AP-induced rats received octreotide vehicle (glacial acetic acid, sodium acetate trihydrate, phenol liquefied, mannitol, and water) at time 0.8 and 16 h after induction. Group E is AP-induced rats received ibudilast vehicle (dimethyl sulfoxide) at 0.8 and 16 h after induction. Group F is AP-induced rats received octreotide at 20 $\mu\text{g}/\text{kg}$ subcutaneously at time 0.8 and 16 h after induction. Group G is AP-induced rats received ibudilast (100 $\mu\text{g}/100\text{ g}$ IP) at time 0.8 and 16 h after induction. Group H is AP-induced rats received the similar doses of both octreotide and ibudilast at time 0.8 and 16 h after induction. Twenty-four hours after the induction, a midline incision was made, and blood samples were withdrawn from the heart using a syringe, the animals were then sacrificed.^[8]

Blood samples and tissue collection

Twenty-four hours after the induction, a midline incision was made, and blood samples were withdrawn directly by a syringe from the heart. The animals were then sacrificed.^[8] The blood was centrifuged at 3000 rpm for 10 min. The resulting serum was kept at -80°C to determine interleukin-1 beta (IL-1 β) levels and serum lipase level using sandwich enzyme-linked immunosorbent assay^[9] and biochemical assay,^[10] respectively, according to manufacturer's instructions. After sacrificing, the pancreas was isolated and placed in 10% formaldehyde for histopathological examination.

Statistical analysis

The collected data were analyzed using the Statistical Analysis Package for the Social Sciences version 20. The analyzed data were expressed as mean \pm standard error. The Pearson correlation coefficient was used to determine the correlation between the two continuous variables.^[11]

RESULTS

The effect on serum IL-1 β level in different study groups

There was no significant difference between serum IL-1 β levels of Groups A and B. A statistically significant increase in the serum IL-1 β level was noted in Groups C, D, and E when compared with the levels of Groups A and B. Groups G and H showed a statistically significant decrease in the serum IL-1 β level when compared with the levels of Groups C, D, and E. No significant difference was seen among rats treated with either combination or individual agents [Figure 1].

The change in serum lipase level in different study groups

There was no significant difference between the serum lipase levels of control Groups A and B. Groups C, D, and E showed a significant increase in the serum lipase level when compared with the levels of control Groups A and B. Treated Groups F, G, and H showed a significant decrease in the serum lipase level when compared with the levels of

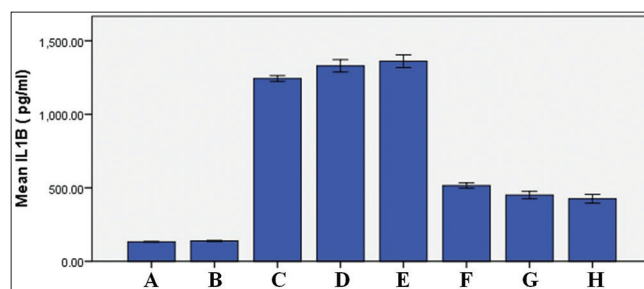


Figure 1: Mean serum interleukin-1 beta (pg/ml) among the study groups

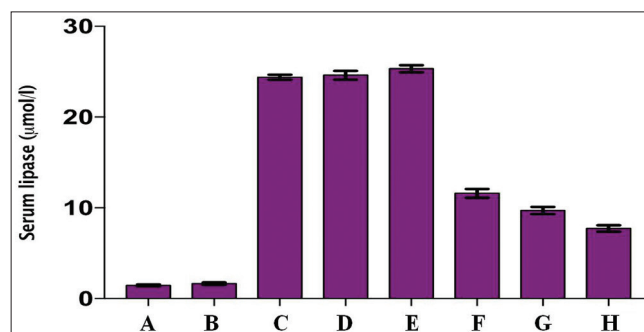


Figure 2: Mean serum lipase ($\mu\text{mol/l}$) among the study groups

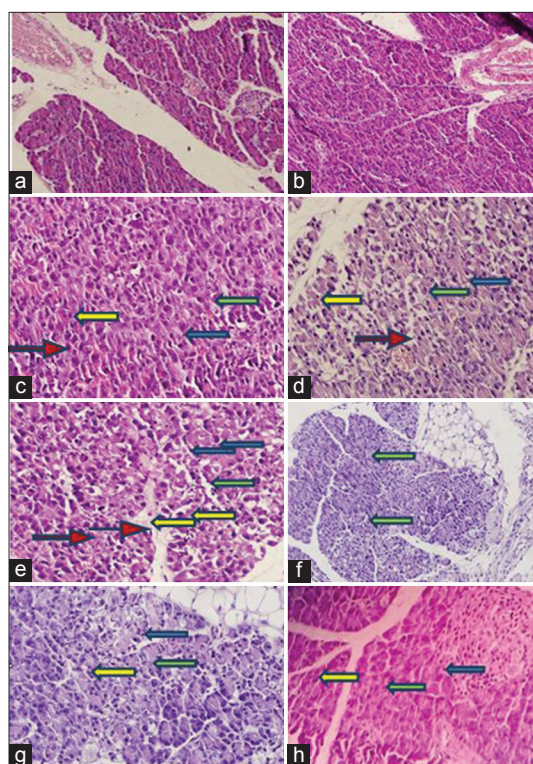


Figure 3: The changes in histopathological picture of pancreas in the study groups. (a) Control group is normal pancreatic tissue; (b) sham group is normal pancreatic tissue, normal acing, no edema, no congestion, no inflammatory cells infiltration, and no degeneration; (c) Acute pancreatitis (AP)-induced group: There is diffused acing, congestion (red arrow), edema (green arrow), mild degeneration (blue arrow), scattered lymphocytes, and inflammatory cell infiltration (yellow arrow); (d) octreotide vehicle group: Congestion (red arrow), edema (green arrow), inflammatory cells infiltration (yellow arrow), and degeneration (blue arrow); (e) dimethyl sulfoxide-treated group: Congestion (red arrow), edema (green arrow), inflammatory cells infiltration (yellow arrow), and degeneration (blue arrow); (f) octreotide group: There is a good response, the pancreatic tissue returned to normal, only focal congestion, partial edema (green arrow); (g) ibudilast-treated group: There is a good response, less edema (green arrow), few inflammatory cells infiltration (yellow arrow), and less degeneration (blue arrow); (h) combination group: Mild edema (green arrow), mild congestion, mild inflammatory cells infiltration (yellow arrow), mild degeneration (blue arrow)

Groups C, D, and E. Rats treated with either ibudilast or in combination with octreotide showed a significant reduction in serum lipase level when compared with rats received octreotide alone [Figures 2 and 3].

Pearson's correlation analysis

Strong positive correlations was observed between the different study parameters like between the IL-1 β , serum lipase, and PW/TBW ratio. This means that when one parameter increases, an increase in the other parameter was also observed [Table 1].

Table 1: Pearson's correlation coefficient between the study parameters

	IL-1 β	Lipase
IL-1 β	1	0.98**
Lipase	0.98**	1

**Correlation is significant at the 0.01 level. IL-1 β : Interleukin-1 beta

DISCUSSION

The effect on serum IL-1 β level in different study groups

In this experimental study, octreotide lowered the level of serum IL-1 β significantly after induction of AP. In 2017, Yang *et al.* announced that octreotide reduces the levels of pro-inflammatory cytokines such as IL-1 β in the serum. At the same year, Yang *et al.* reported the same result.^[12] Ibadi *et al.*, in 2018, revealed similar result;^[13] in 2018, a study has showed the same result of decreasing IL-1 β when octreotide was used after AP induction in rats.^[14] This action of octreotide was through anti-inflammatory effect by reducing leukocyte infiltration and reducing pro-inflammatory cytokines. This study obtained a result of decreasing serum level of IL-1 β by ibudilast in rats after induction of AP by L-arginine. To the best of our knowledge, this study is the first one that deals with the effect of ibudilast in AP. This action of ibudilast was probably through PDE inhibition, so inhibits cAMP response element binding and activating transcription factor 1 which stimulate pro-inflammatory cytokines or may be through inhibition of mitogen-activated protein kinases so inhibition of NF- κ B which stimulates the pro-inflammatory cytokines. The combination of octreotide and ibudilast decreased IL-1 β significantly as compared with AP and solvents groups. No significant difference when it was compared with the usage of ibudilast alone, but it decreased IL-1 β significantly more than octreotide alone.

The effect on serum lipase level in different study groups

This study had shown that the level of serum lipase was lowered significantly when octreotide used in rats with induced AP. In 2014, Er and Zhi reported that octreotide lowered serum lipase in case of AP.^[15] Cakir *et al.*, in 2015, uphold this result. They stated that octreotide alone or in combination with diclofenac causes a noticeable decrease in serum lipase when used, but they prefer diclofenac over octreotide.^[16] Ibadi *et al.* (2018) indicated the same result.^[13] This effect of octreotide is through its antisecretory action either directly on lipase or through the prevention of trypsinogen secretion which stimulates lipase. When ibudilast is used in rats after induction of AP serum level of lipase enzyme decreased significantly. This action may be by attenuating the acinar cell damage and inhibition

of trypsinogen which stimulates lipase secretion. The combination of octreotide and ibudilast lowered the serum lipase level significantly after AP induction. There is a significant difference when compared with octreotide alone as the combination decreased lipase more significantly and no significant difference with ibudilast alone.

The effect on pancreatic histology in different study groups

Histopathological examination of the pancreatic tissue in rats which given octreotide after induction of AP with L-arginine revealed improvement of histopathological changes such as decreasing of edema, congestion, inflammatory cells infiltration, and degeneration. These findings were noted in the present study. Wenger *et al.*, in 2007, said that the early hour's octreotide administration reduces the severity of histopathological changes in AP.^[6] In 2015, Cakir *et al.* concluded that the edema, hemorrhage, parenchymal necrosis, fat necrosis, leukocyte infiltration, and fibrosis that present in the pancreatic tissue in AP were significantly reduced when using octreotide.^[16] The histopathological changes that occurred due to AP induction with L-arginine were mitigated when ibudilast was used. Edema is decreased, congestion is decreased, inflammatory cells infiltration is diminished, and degeneration is decreased significantly. This action may be due to inhibition of free radical formation which prevents acinar cells injury, prevents cells and fluid leakage and so prevent edema, congestion and other histopathological changes, or may be through anti-inflammatory action as PDE inhibitor action as discussed above, or as TLR4 antagonism and decreases NF- κ B so decreases cell damage and inflammatory cells infiltration and decreases, acinar cell necrosis, edema, and hemorrhage. The using of drug combination after AP induction significantly decreased edema, congestion, inflammatory cells infiltration, and degeneration, but the decrease was less than if we use each one drug alone. The cause is not determined and may be interaction between the two drugs or side effect of one or both drugs.

CONCLUSION

It was concluded that ibudilast and octreotide can significantly attenuate the local and systemic effect of AP. The efficacy of ibudilast and octreotide are nearly the same. Their use as the combination has no preferential effect compared with each drug used alone. Thus these drugs can be used effectively in cases of pancreatitis as it leads to high morbidity and mortality.

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