Ibudilast and octreotide can ameliorate acute pancreatitis via down-regulation of the inflammatory cytokines and Nuclear Factor- Kappa B expression

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Abstract

Background: Acute pancreatitis (AP) is severe inflammation of the pancreas that can be of two major types: mild AP and severe AP. In this case, some pancreatic cells can produce various pro-inflammatory cytokines and transcription factors thereby affecting the function of pancreas.

Objective: To study the efficacy of Ibudilast in comparison with octreotide, in a rat model of AP.

Methods: A total of 48 male rats were randomly divided into 8 groups (6 per each). AP was induced by L-arginine model which has a high reproducibility. Octreotide and Ibudilast were administered individually or in combination at 0, 8 or 16 hr after induction. After 24 hr of treatment, each rat was weighed and blood samples were collected for measurement of biochemical markers, and then the pancreas was extracted to obtain the pancreatic weight to the total body weight (PW/TBW) ratio and for vertical gel electrophoresis experiments.

Results: In both octreotide-treated group and ibudilast-treated group, there were statistically significant decrease in serum pro-calcitonin (PCT) and tumor necrosis factor-alpha (TNF-α) when compared to control group or sham group, as well as, a decrease in PW/TBW ratio and in nuclear factor kappa B (NF-κB) protein level using vertical gel electrophoresis. However, in octreotide + ibudilast-treated group there was no statistically significant difference when compared with groups treated with either agents.

Conclusion: Ibudilast and octreotide can significantly attenuate the local and systemic effect of AP. The efficacy of ibudilast and octreotide are almost the same. Co-treatment of rats with both Ibudilast and octreotide has no preferential effect when compared with rats treated with the individual agent.

Keywords: Ibudilast, octreotide, acute pancreatitis, inflammatory cytokines.

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Introduction

Acute pancreatitis (AP) is severe inflammation of the pancreas. Most patients with AP are diagnosed with a mild AP that has a mortality rate of less than 1% and is usually self-limiting. However, 10% - 20% of the AP cases are diagnosed with severe AP that has a 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) \(^2\). NF-κB has a vital part in AP. It is the transcriptional agent that regulates many genes, including those which are responsible for inflammation, tissue damage and healing \(^3\).

Ibudilast is a non-selective cAMP phosphodiesterase (PDE) inhibitor. It inhibits the formation of the nitric oxide and reduces ROS. As a TLR4 antagonist, it has anti-inflammatory and pain modulating effects \(^4\). As a PDE inhibitor, it stimulates the anti-inflammatory cytokines and prevents NF-κB 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 also prevents insulin, glucagon, amylase, trypsin, lipase enzyme, thyroid stimulating hormone and growth hormone release \(^6\). These secretions play a vital 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 by altering metabolism of ROS \(^7\).

By discerning the multiple varied effects of ibudilast especially focusing on anti-inflammatory effects we hypothesized that it could be beneficial in animal models of AP. Thus, the aim of this study was to demonstrate the efficacy of ibudilast in comparison with octreotide in a rat model of AP.

**Materials and Methods**

**Experimental animals:** The study was performed according to the America guidelines of animal experimentation. A total of 48 adult male Sprague Dawley rats weighing 250–300 g were obtained from certified animal house. They were placed in a 12-hour light and 12-hour dark cycles, at a temperature of 22±2°C and humidity of 60%–65% and were fed standard pellet diet *ad libitum*. Ethical permission was taken from Institutional Ethics Committee Kufa University, Iraq letter number 1001-2018.

**Study design:** Forty eight rats were randomly divided into 8 groups (A through H, 6 rats per each group). Group A is control group, where rats were not subjected to any intervention. Group B is sham group, where rats were receiving 1.25 mL normal saline/100 g (body weight) intraperitoneally (IP). Group C is AP-induced rats receiving 2 doses of L-arginine at 250 mg/100 g body weight IP with 1 h gap between injections. Group D is AP-induced rats receiving octreotide vehicle (Glacial acetic acid, sodium acetate trihydrate, phenoliqued, mannitol and water) at 0, 8 and 16 hr after induction. Group E is AP-induced rats receiving ibudilast vehicle (dimethyl sulfoxide, DMSO) at 0, 8 and 16 hr after induction. Group F is AP-induced rats + octreotide at a dose of 20µg/kg subcutaneously at 0, 8 and 16 hr after induction. Group G is AP-induced rats receiving ibudilast at a dose of 100 µg/100g IP at 0, 8 and 16 hr after induction. Group H is AP-induced rats receiving both octreotide and ibudilast at 0, 8 and 16 hr after induction.

**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 PCT and TNF-α levels according to manufacturer’s instructions \(^9\) until use. After sacrificing, the pancreas was isolated and cleaned from any fatty tissue and weighed for the determination the PW/TBW ratio and part of it was kept at -80°C to determine NF-κB protein level using vertical gel electrophoresis \(^10\).

**Statistical analysis:** Data were analyzed by using SPSS version 20. The analyzed data were expressed as mean ± SEM. The Pearson correlation coefficient was used to determine the correlation between the two continuous variables \(^11\).

**Results**

The changes in serum TNF-α level among study groups:

There was no statistically significant difference between serum TNF-α levels of group A and group B. A statistically significant increase in the serum TNF-α level was noted in groups C, D and E when compared to that of groups A and B. Groups F, G and H showed a statistically significant decrease in the serum TNF-α
level when compared to that of groups C, D and E. Furthermore, no significant difference was seen among rats treated with either combination or individual agents (Figure 1).

The changes in serum PCT level among study groups:
There was no statistically significant difference between the serum PCT levels of group A and group B. Groups C, D and E showed a statistically significant increase in serum PCT level when compared to that of groups A and B. Groups F, G and H showed a statistically significant decrease in serum PCT level when compared to that of groups C, D and E. Furthermore, no significant difference in PCT levels was seen among rats treated with either combination or individual agents (Figure 2).

The effect on the PW/TBW ratio in different study groups:
PW/TBW ratio of group A and B revealed no significant difference. Group C, D and E showed a statistically significant increase in the PW/TBW ratio when compared to group A and B. Group F, G and H showed a statistically significant decrease in the PW/TBW ratio when compared to that of groups C, D, and E. There were no changes among rats treated with either individual agents or co-treatment (Figure 3).
The changes in NF-κB protein levels among study groups:
Groups A and B had no significant difference between them for the NF-κB protein band. Group C, D, and E showed a significant increase in the NF-κB protein band compared with Groups A and B. Groups F, G, and H showed a significant decrease in the NF-κB protein level when compared to that of groups C, D, and E; and no significant differences were noted among them (Figure 4)
Correlation of TNF-α, PCT and PW/TBW Ratio
Strong positive correlation was observed among TNF-α, PCT and PW/TBW Ratio (Table 1).

Table 1: Pearson correlation coefficient among study parameters

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<th>TNF-α</th>
<th>PCT</th>
<th>PW/TBW Ratio</th>
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<tbody>
<tr>
<td>TNF-α</td>
<td>1</td>
<td>0.95**</td>
<td>0.9**</td>
</tr>
<tr>
<td>PCT</td>
<td>0.95**</td>
<td>1</td>
<td>0.93**</td>
</tr>
<tr>
<td>PW/TBW Ratio</td>
<td>0.9</td>
<td>0.93**</td>
<td>1</td>
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*Correlation is significant at the 0.01 level.

Discussion
The effect on serum TNF-α level in different study groups:
Data in this study demonstrated that rats that received octreotide showed a statistically significant decrease in the serum TNF-α level when compared to vehicle-treated rats. Similar results were obtained by Zhongguo et al. in 2015, who reported that the use of octreotide decreases TNF-α level which mitigates the pancreatic injuries and increases the survival rate in induced AP animals (12). In Ibudilast-treated rats, the serum TNF-α level was reduced significantly when compared to vehicle-treated rats. Co-treatment of rats with both octreotide and ibudilast also resulted in a statistically significant decrease in the TNF-α levels when compared to control groups. Data from rats co-treated with both agents were not significantly different from that obtained from rats treated with single agent. As per our knowledge, this study is unique in identifying the effect of ibudilast and combination of octreotide and ibudilastin AP.

The effect on serum PCT level in different study groups:
In rats that received octreotide, serum PCT level was lowered significantly when compared to vehicle-treated rats. Dheeraj K Gandotra et al in 2004\(^\text{[13]}\) and Y. Jin et al. in 2018 also reported similar results\(^\text{[14]}\). In ibudilast-treated rats, serum PCT level decreased significantly when compared to that observed in vehicle-treated group. Co-treatment with octreotide and ibudilast also significantly decreased serum PCT level when compared to control groups, although this combination was unable to decrease PCT to the levels less than that driven by single agents. The actions of ibudilast on the serum TNF-α and PCT level were postulated to PDE inhibition that further inhibits pro-inflammatory cytokines. Alternatively, the action might occur due to inhibition of mitogen-activated protein kinase (MAPK) that results in the inhibition of NF-κB, the major factor involved in the stimulation of the pro-inflammatory cytokines.

**The effect on the PW/TBW ratio in different study groups:**

Rats treated with octreotide showed a significant reduction in the PW/TBW ratio. Zong-Guang Zhou and co-workers in 2002 reported a similar finding when they used octreotide \(^\text{[15]}\). The PW/TBW ratio also significantly decreased in ibudilast-treated group. This decrease in the PW/TBW ratio was attributed to diminished edema and reduced leakage of fluid from blood vessels in the pancreas. The combination of octreotide and ibudilast also significantly decreased the PW/TBW ratio in the AP-induced rats. Co-treatment was unable to decrease PW/TBW ratio to levels induced by individual agents.

**The effect on NF-κB expression in different study groups:**

There is a decrease in NF-κB protein expression in pancreatic tissue when octreotide was used after AP induction. Octreotide prevents the production of various cytokines, especially TNF-α and IL-6, and reduces the formation of ROS. NF-κB plays a vital role in the production of cytokines and is stimulated by ROS so that octreotide can indirectly reduce cytokines by inhibiting ROS\(^\text{[16]}\). Liang et al. in 2008, used octreotide in AP, they noted that, there was a decrease in NF-κB protein expression and a decrease of cell content secretion and so a decrease in acinar cell death\(^\text{[17]}\). During 2012, Jingmin et al. found that octreotide had a protective effect on rat’s brain by decreasing NF-κB protein so decreasing pancreatic and brain cell death\(^\text{[18]}\). Octreotide is beneficial in the case of AP and reduces the severity of the disease and mortality by preventing the stimulation of NF-κB\(^\text{[19]}\). The expression of NF-κB in pancreatic tissue was lowered by ibudilast in AP-induced rats. This action may be through inhibition of MAPK and thus, the inhibition of NF-κB or may be due to inhibiting oxidative stress which stimulates the NF-κB formation. Other possibility is via inhibition of TLR4 signaling pathway in which NF-κB activation is a downstream event.

**Conclusion**

To conclude, ibudilast and octreotide can significantly attenuate the local and systemic effect of AP. The efficacy of both ibudilast and octreotide was more or less the same. Co-administration of both agents has no preferential effect when compared to administration of these agents individually. Thus, these drugs can be used effectively to decrease high morbidity and mortality associated with pancreatitis.

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