EXPERIMENTAL STUDY

Intraductal botulinum toxin injection suppressed the inflammation in experimental acute pancreatitis

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ABSTRACT

BACKGROUND: Acute pancreatitis (AP) is inflammation of pancreas in which pancreas enzymatic activity is increased. Parasympathetic innervation of pancreas plays an important role in several functions of pancreas. Botulinum toxin (BTx) might be a tool to suppress the pancreas activity in AP.

METHODS: In the preliminary experimental study, BTx (15U/kg) was administered directly and intraductal ways. After 10 days, blood amylase, lipase, trypsinogen, insulin, and glucagon levels were compared and no significant difference was seen between groups. Intraductal BTx administration is preferred for experimental AP model in rats; control, AP, intraductal BTx, and AP with Intraductal BTx (AP+BTx). AP was created by intraperitoneal injection of cerulean 20 μg/kg/injection (5 times). After 24 h, serum amylase, lipase, IL-6, IL-1β, TNF-α, and IL-10 were measured and pancreas tissue was evaluated for inflammation and necrosis.

RESULTS: Mean serum amylase, lipase IL-6, IL-1β, and TNF-α levels of the AP group were significantly higher compared to the other groups ($p<0.05$). However, there was no significant difference between the amylase and lipase levels of control, BTx, and AP+BTx groups. Serum insulin and glucagon levels in AP group were significantly higher than control and BTx groups ($p<0.05$). However, there is no significant difference between the insulin and glucagon levels of AP and AP+BTx groups. in pathological evaluation. In AP+ BTx group, there is less amount of centrilobular necrosis and there is mild inflammation and hyperplasia of pancreatic duct epithelium.

CONCLUSION: Administration of intraductal BTx suppressed the AP without making significant suppression in endogenous activity of pancreas.

Keywords: Acetylcholine; acute pancreatitis; botulinum toxin; cerulein; pancreas duct.

INTRODUCTION

Acute pancreatitis (AP) is the sudden onset of inflammation of the pancreas. This condition is believed to be caused by the upregulated activity of trypsin with pancreatic acinar cells. Enzyme activation within the pancreas leads to its autodigestion, followed by local inflammation.[1] The inhibition of pancreatic activity appears to be a reasonable first step in treatment. Blockage or inhibition of activated enzymes has been investigated in AP, and so has the inhibition of exocrine pancreatic activity, but the results are controversial.[1–4]

The exocrine function of the pancreas is controlled by both the neuronal and humoral systems.[5,6] Parasympathetic nerve

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activation of the pancreas (e.g., by food intake) plays a major role in stimulating enzyme secretion from the exocrine pancreas. Interestingly, muscarinic cholinergic mechanisms may also take part in the pathogenesis of pancreatitis.[5,7] Neuronal control depends on cholecystokinin (CCK), which is a major secretagogue and a trophic factor for the pancreatic epithelium. CCK stimulates receptors on vagal efferent fibers of intrapancreatic neurons, as well as acetylcholine (Ach) secretion by pancreas stellate cells, which induces amylase secretion.[8]

Botulinum toxin (BTx) inhibits the release of acetylcholine from cholinergic nerves.[9] This function results in neuromuscular paralysis and inhibition of the cholinergic system. BTx has been used for its paralytic effect on voluntary muscles in the treatment of conditions such as strabismus and dystonia, as well as diseases related to smooth muscle. In addition, BTx also has several effects on hormonal systems, including insulin, growth hormone, and ADH-related renal functions.[9–12] Thus, the aim of this study is to show the biochemical and histological effects of BTx in an experimental model of AP.

MATERIALS AND METHODS

The design of our study was approved by the Animal Experiments Ethics Committee of Kocaeli University (KOU HADYEK 9/4-2015). A total of 62 male Wistar Albino rats (300–400 g) were used for the study. All rats were fed with a standard pellet diet and tap water ad libitum. They were kept in cages on a 12-h dark/12-h light cycle at controlled temperature (20–22°C). The food was withdrawn 12 h before the experiment. All of the surgeries were performed after an intramuscular injection of 40 mg/kg of ketamine hydrochloride (Ketalar, Parke Davis, Eczacıbasi, Istanbul, Turkey) and 5 mg/kg of xylazine (Rompum, Bayer AG, Leverkusen, Germany), and all efforts were made to minimize suffering.

We first performed a preliminary experiment to determine the administration route of BTx. In this preliminary experiment, we aimed to determine whether there is any significant suppression of endogenous secretion of the pancreas and whether there is a difference between local and intraductal application of BTx. In the preliminary study, 30 rats were randomly divided into three equal groups: A control group, a local BTx group, and an intraductal BTx group. All rats in each group were operated on through a midline incision under aseptic conditions.

In the local BTx group, the pancreas was revealed and 15 U/kg of BTx (Allergan, Dublin, Ireland) was injected around the pancreas. The dose was selected as previous experimental studies.[13] In the intraductal BTx groups, the pancreas was exteriorized, the proximal bile duct was clamped at the level of the liver hilum, and the distal bile duct was cannulated using a 19-gauge polyethylene catheter through the duodenal wall. 15 U/kg of BTx was then introduced by intraductal retrograde injection.

In the control group, the pancreas was exteriorized, the proximal bile duct was clamped at the level of the liver hilum, and the distal bile duct was cannulated using a 19-gauge polyethylene catheter through the duodenal wall. Only 1 ml of saline was introduced by intraductal retrograde injection. After ensuring, there was no active bleeding, the abdomen was closed in layers. Rats were monitored for 10 days to check for any changes in habits. Tail blood sugar was measured daily by a portable glucometer (Accu-Chek, Roche, Basel, Switzerland). After 10 days, rats in all groups underwent reoperation to collect samples. After blood sampling, the pancreas was carefully dissected from its surrounding attachments and excised. All rats were killed by hemorrhage. Blood levels of amylase, lipase, trypsinogen, insulin, and glucagon were measured by a commercial solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) kit (Elabscience Biotechnology Co, Wuhan, P.R.C.). The results are given in Table 1. Pancreatic edema, inflammation, and necrosis were evaluated according to the study by Schmidt et al.[14] The inflammation scoring details were given in Table 2.

The results showed that neither local nor intraductal BTx application resulted in any suppression of endogenous hormone secretion of the pancreas. The mean glucose levels in the tail blood of the control, local BTx, and intraductal BTX groups were 7.8, 8.4, and 8.6 mmol/ml, respectively ($p>0.05$). The rats were healthy during follow-up. Only the serum levels of glucagon in the intraductal BTx group were significantly lower than in the control groups.

In the pathological evaluation, the edema scores of the control, local BTx, and intraductal BTx groups were 0.8, 1.3, and 1.2, respectively ($p>0.05$). The pathological inflammatory infiltrate scores of the control, local BTx, and intraductal BTx groups were 0.5, 1.0, and 1.1, respectively ($p>0.05$). There was no pancreatic tissue necrosis, perivascular inflammation, or hemorrhage in any of the groups. The preliminary results did not show any harmful effects of BTx on the pancreas and

Table 1. Serum amilase, lipase, trypsinogen, insulin and glucagon levels (mean±SEM) among groups

	Amilase (ng/mL)	Lipase (pg/mL)	Trypsinogen (ng/mL)	Insulin (ng/mL)	Glucagon (pg/mL)
Control (n=10)	13.6±3.2	10.1±2.4	15.2±0.6	5.9±0.4	398.8±125.3
Local BTx (n=10)	16.5±4.3	8.9±2.8	15.4±0.8	4.4±1.1	423.4±112.2
Intraductal BTx (n=10)	17.7±4.8	11.4±2.6	14.3±0.9	4.5±1.0	364.3±98.5

*ANOVA test is used. P>0.05. BTx: Botulinum toxin.

Table 2. Histopathological Scoring System[14]

Scores	Edema	Acinar necrosis	Hemorrhage and fat necrosis	Inflammation and perivascular infiltrate
0	Absent	Absent	Absent	0–1 intralobular or perivascular leukocytes/ HPF
0.5	Focal expansion of interlobar septae	Focal occurrence of 1-4 necrotic cells/HPF	1 focus	2–5 intralobular or perivascular leukocytes/ HPF
1	Diffuse expansion of interlobar septae	Diffuse occurrence of 1-4 necrotic cells/HPF	2 foci	6–10 intralobular or perivascular leukocytes/ HPF
1.5	Same as 1+ Focal expansion of interlobular septae	Same as 1 + focal occurrence of 5–10 necrotic cells/HPF	3 foci	11–15 intralobular or perivascular leukocytes/ HPF
2	Same as 1+ diffuse expansion of interlobular septae	Diffuse occurrence of 5–10 necrotic cells/HPF	4 foci	16–20 intralobular or perivascular leukocytes/ HPF
2.5	Same as 2+ Focal expansion of interaciner septae	Same as 2 + focal occurrence of 11–16 necrotic cells/HPF	5 foci	21–25 intralobular or perivascular leukocytes/ HPF
3	Same as 2+ diffuse expansion of interaciner septae	Diffuse occurrence of 1 1–16 necrotic cells/HPF (foci of confluent necrosis)	6 foci	26–30 intralobular or perivascular leukocytes/ HPF
3.5	Same as 3+ Focal expansion of intercellular spaces	Same as 3 + focal occurrence of >16 necrotic cells/HPF	7 foci	>30 leukocytes/HPF or focal microabscesses
4	Same as 3+ diffuse expansion of intercellular spaces	> 16 necrotic cells/HPF (Extensive confluent necrosis)	≥ 8 foci	>35 leukocytes/HPF or confluent microabscesses

HPF: High-power field. This table is taken from Schmidt J et al.[14]

the rats. Thus, we decided to perform an AP experiment involving the intraductal administration of BTx since intraductal administration can be used in clinical settings.

A total of 32 rats were randomly divided into four groups: Control, AP, intraductal BTx, (BTx), and AP+BTx groups. The control group underwent only laparotomy with a midline incision. AP was induced by five intraperitoneal injections of cerulein at a dose of 20 μg/kg/injection at 1-h intervals (Sigma-Aldrich Chemical, Steinheim, Germany). 4 ml of saline were injected subcutaneously in the flank region of all control rats.

In the BTx group, the experiment was performed as in the preliminary study. In the AP+BTx group, 1 h after the last cerulein injection, the rats underwent laparotomy for intraductal BTx administration (15 U/kg). After the operation, the abdomen was closed. All rats were permitted to have water and food after the experiment. Food intake was ceased 2 h before being sacrificed. All rats were operated on under anesthesia after 24 h. Laparotomy was performed by midline incision. After blood withdrawal, the pancreas was carefully dissected from its attachments to the stomach and excised. All rats were killed by hemorrhage.

Serum amylase, lipase, IL-6, IL-1β, TNF-α, and IL-10 were measured by ELISA using commercial kits (Elabscience Biotechnology Co., Wuhan, P.R.C.). Pancreas tissue inflammation and necrosis scores were evaluated by pathological examination as described by Schmidt et al.[14] Samples of pancreatic tissue were fixed in 10% formalin, processed, and embedded in paraffin. Serial sections were stained with hematoxylin and eosin. The slides were examined by an experienced pathologist who had no knowledge of the group assignments.

The data are expressed as the mean±SEM. Comparisons within groups were made using the Mann–Whitney U test. One-way ANOVA and a post hoc Tukey test were carried out for multiple comparisons. $P<0.05$ was considered statistically significant. The statistical analyses were performed using the software SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

During the experiments, one rat each from the AP and AP+IDBTx groups was replaced by additional rats. The mean serum levels of amylase and lipase in the AP group were significantly higher than in the other groups ($p<0.05$). However, there was no significant difference between the amylase and lipase levels of the control, BTx, and AP+BTx groups ($p>0.05$) (Fig. 1). Serum levels of insulin and glucagon in the AP group were significantly higher than in the control and BTx groups ($p<0.05$). However, there was no significant difference be-

tween the insulin and glucagon levels of the AP and AP+BTx groups (p>0.05) (Fig. 2).

The mean serum levels of IL-6, IL-1β, and TNF-α in the AP groups were significantly higher than in the other groups (p<0.05). However, there was no significant difference between the serum levels of IL-6, IL-1β, and TNF-α among the control, BTX, and AP+BTx groups (p>0.05) (Fig. 3). Serum levels of IL-10 in the AP groups were significantly lower than in the control, BTx, and AP+BTX groups (p<0.05) (Fig. 3).

The pathological inflammatory scores of groups were given in Table 3. Edema, acinar necrosis, inflammatory infiltrate, hemorrhage, fat necrosis, and perivascular inflammation scores of AP group were significantly higher than control group (p<0.05). There was no hemorrhage, fat necrosis, and perivascular inflammation in the control group (Fig. 4). There was significant edema, acinar necrosis, and inflammatory infiltrate in the AP group (Fig. 5). Edema, acinar necrosis, and inflammatory infiltrate scores of AP group were significantly higher than BTx group (p=0.02). Edema and inflammatory infiltrate scores of AP group were significantly higher than AP+BTx group (p=0.03). In the AP+BTx group, there was less aciner necrosis, and there was mild inflammation and hyperplasia of the pancreatic duct epithelium when compared with AP group (Fig. 6). In the BTX group, there was edema around the main pancreatic ducts (Fig. 7).

Table 3. Histopathological scores of the groups

	Control (n=8)†	AP (n=8)†	BTx (n=8)	AP+BTx (n=8)
Edema	0.8	2.5*,§	1.2§	1.8*
Acınar necrosis	0.2	1.8§	0.8§	1.2
Inflammatory infiltrate	0.2	2.6α,§	0.8§	1.8α
Hemorrhage	0	1.6	0.3	0.8
Fat necrosis	0	1.2	0.5	0.8
Perivascular inflammation	0	1.4	0.4	0.9

†P<0.05; Between control group and AP group. *P=0.003; Edema score between AP and AP+BTx groups. αP=0.04; Inflammatory infiltrate score between AP and AP+BTx groups. §P=0.001; Edema, Acinar necrosis and inflammatory infiltrate scores between AP and BTx groups.

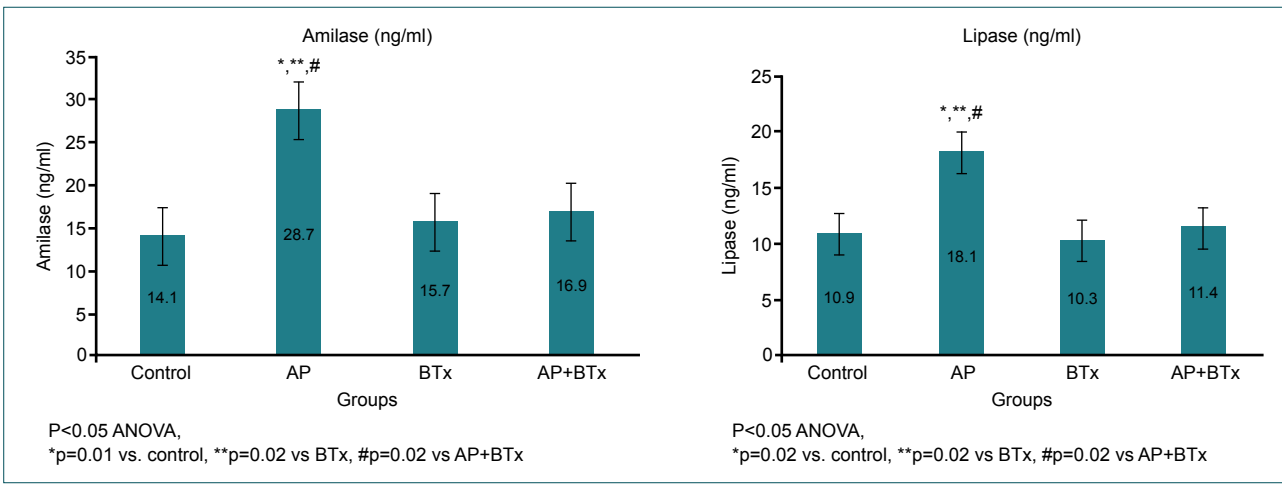

Figure 1. Mean serum levels of amylase and lipase of control, AP, BTx, and AP+BTx groups.

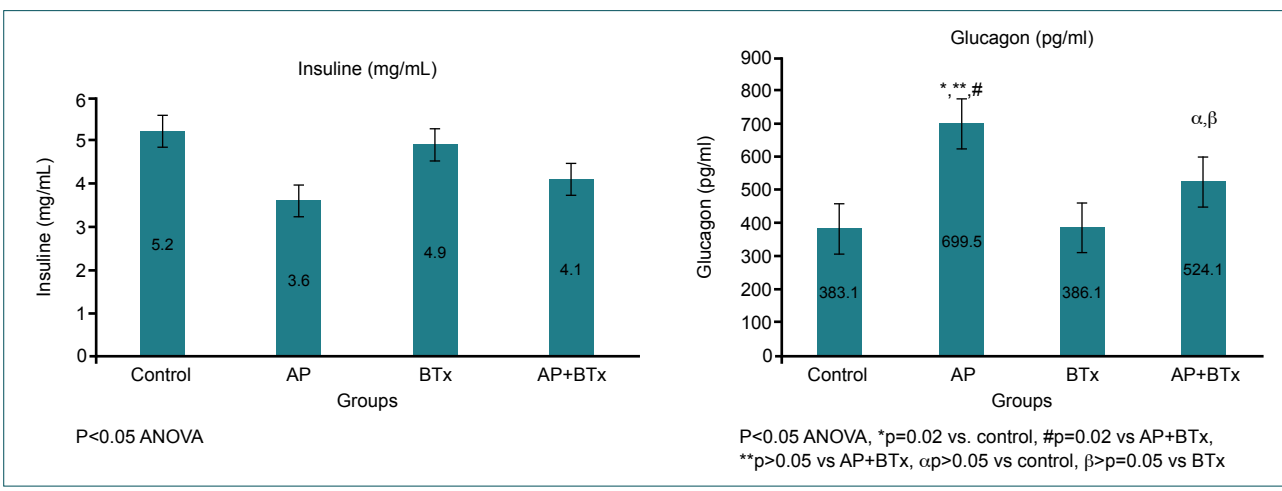

Figure 2. Mean serum levels of insülin and glucagon of Control, AP, BTx and AP+BTx groups.

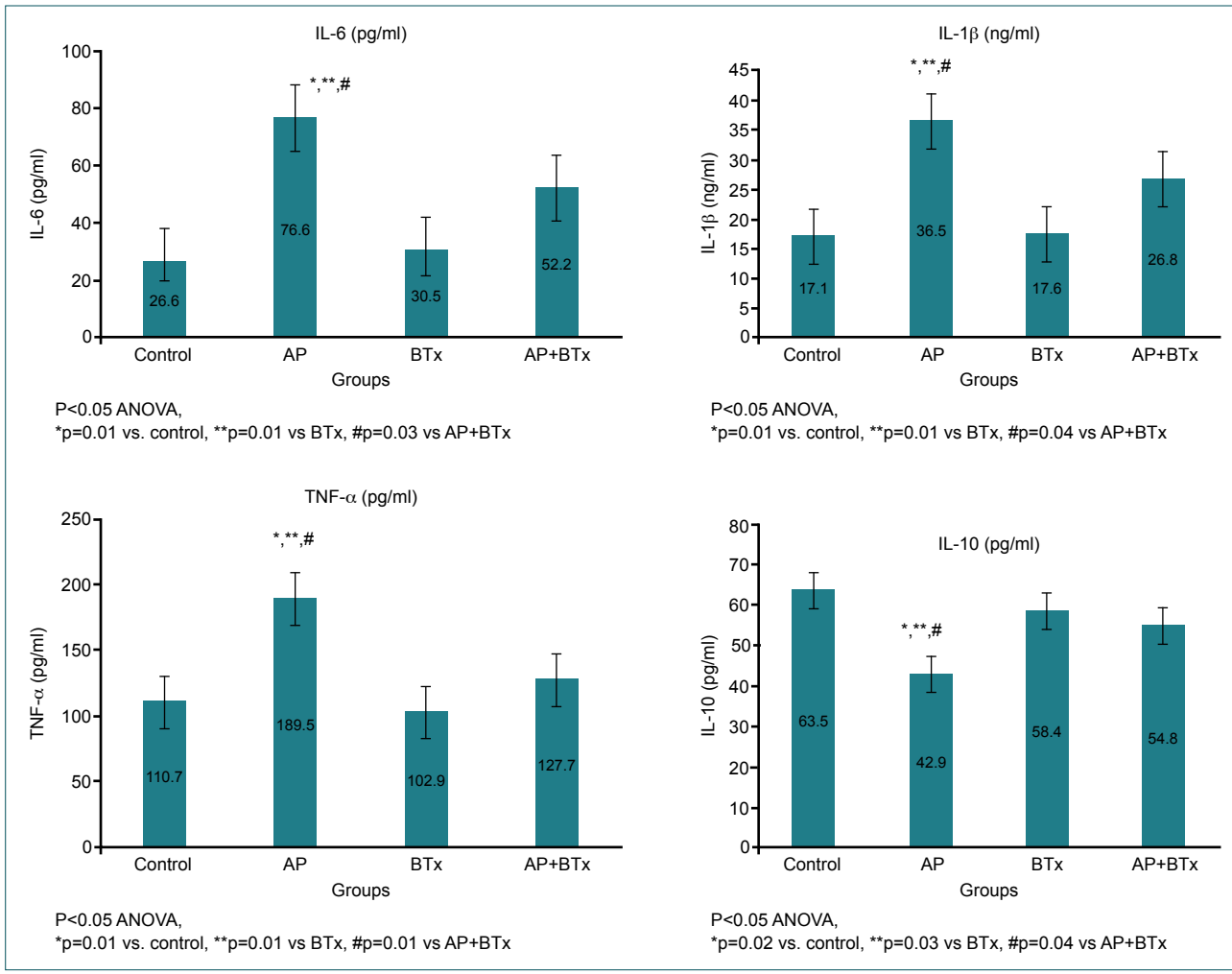

Figure 3. Mean serum levels of IL-6,IL-1β, TNF-α, and IL-10 of Control,AP, BTx and AP+BTx groups.

DISCUSSION

Pancreas secretion is controlled by both the enteric nervous system and intrapancreatic nervous system. Many aspects of the physiology of the pancreas are regulated by cholinergic inputs to the pancreas from preganglionic fibers in both the sympathetic and parasympathetic systems and postganglionic fibers emanating from intrapancreatic ganglia.[15,16] The intrapancreatic ganglia are the integration centers of the pancreatic exocrine secretions and terminal axons from these ganglia sur-

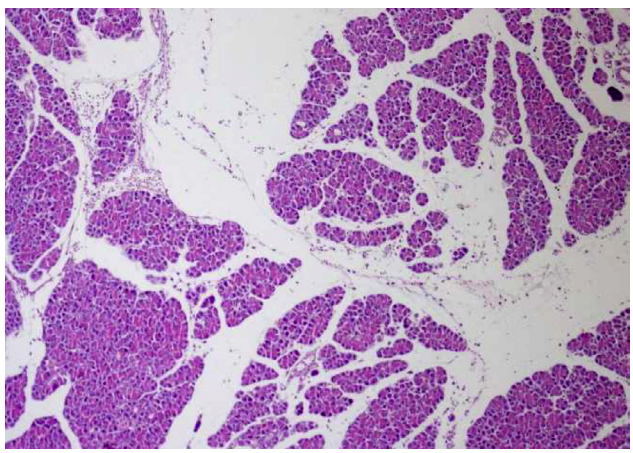

Figure 4. Macroscopic view of pancreas of control group. (H &E,x40). Benign pancreatic tissue. Aside from slight interstitial oedema, noevidence of cellular damage wasobserved.

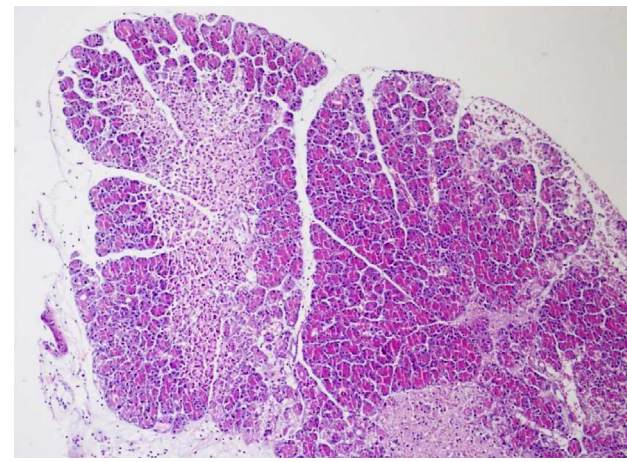

Figure 5. Macroscopic view of pancreas of AP group. (H &E, x100). Pancreatic tissue demonstrating extensive centrilobular coagulative necrosis and acute inflammatory cell infiltration.

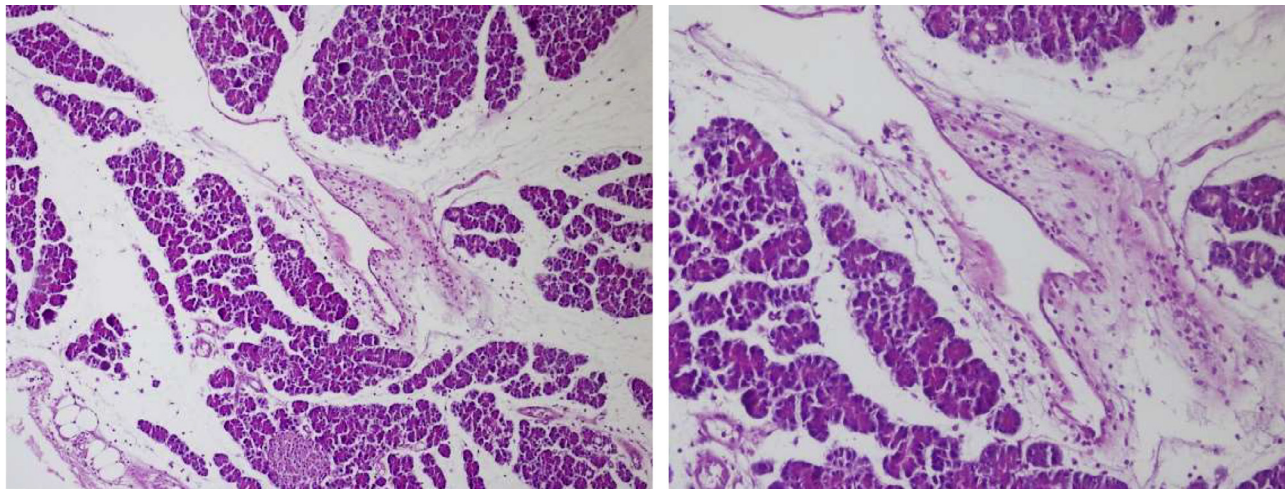

Figure 6. Macroscopic view of pancreas of AP+BT group. (H &E,x100 and x200). Oedema and mild acute inflammation around the main-pancreatic duct. Larger magnification of periductal area. Relatively mild infiltration of neutrophils can be seen.

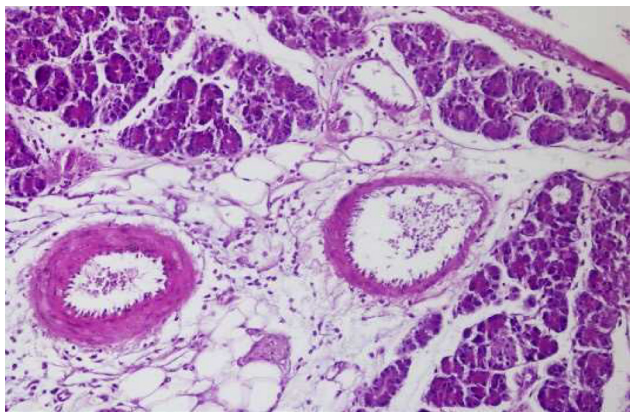

Figure 7. Macroscopic view of pancreas of BTx group. (H &E,x200).Oedema in connective tissue surrounding the main pancreatic vasculature.

round every acinus. Studies on humans and experimental animals have demonstrated that the cholinergic system regulates pancreatic exocrine secretion through vago-vagal reflexes.[15–17] These stimulations are controlled by both the cholinergic system and acetylcholine. Furthermore, the intrapancreatic nervous system enables a degree of independence of the pancreas after vagotomy and is separated from the gut.

In animal models, neither atropine nor vagotomy had any significant effect on the pancreatic secretory response to exogenous CCK.[18] Animal models showed direct stimulatory effects of CCK on the pancreas's acinar cells through special receptors, but there are debates among human studies about whether the human pancreas's acinar cells lack functional CCK receptors.[19,20] However, there is wide acceptance of the concept that CCK-mediated secretion of pancreas enzymes occurs at least in part through an indirect pathway. In this pathway, CCK stimulates receptors on vagal afferent fibers, leading to signals being transmitted through vagal efferent fibers to intrapancreatic neurons. Besides pancreas acinar stimulation, pancreatic stellate cells were shown to have the cellular systems required for the synthesis of Ach, to synthesize and secrete Ach, to stimulate acinar amylase secretion, to express CCK receptors, and to respond to CCK through the stimulation of Ach secretion.[8] The importance of the cholinergic system in the physiology of the pancreas has also been shown in a pancreatitis model.[7,21]

Excessive stimulation of the exocrine pancreas worsens AP and is thus the rationale for using anti-secretory agents as potential therapies for AP. Several agents have been used for the suppression of pancreatic enzymes in AP.[22] BTx is a potent inhibitor of Ach release from pre-synaptic nerve endings and could be a target for the suppression of pancreatitis. The application of BTx to the sphincter of Oddi in recurrent pancreatitis is a safe method and has had nearly 80% in treating spastic sphincters of Oddi.[23] From a technical point of view, endoscopic injection of BTx to the sphincter of Oddi is known to be a safe and feasible method for treating dysfunctions of the sphincter.[23] However, direct application to the pancreas has not been studied.

In our study, neither local nor transduodenal pancreatic duct application showed any suppression of endogenous pancreas secretions such as insulin, glucagon, and other hormones, such as trypsinogen, amylase, and lipase. This showed that BTx application is safe and can be used without fear of endogenous pancreas suppression. Furthermore, intraductal application can be performed in a minimally invasive manner and has similar results to those of local application. However, in this preliminary study, the effects of BTx on exogenous secretion of the pancreas were not studied and will be examined in a later study.

After the preliminary study, our experiment showed that the application of intraductal BTx significantly decreased levels of inflammatory cytokines. TNF-α and IL-1β are known as very significant factors that enhance local tissue destruction, produce distant organ complications, and increase the overall

mortality rate of AP. TNF-α and IL-1β lead to tissue injury by upregulating the levels of adhesion molecules, producing nitric oxide, and activating inflammatory cells to release other cytokines and free oxygen radicals. The administration of BTx decreased the levels of inflammatory cytokines to nearly that of the control group. Anti-inflammatory cytokine IL-10 was significantly decreased in the AP group in comparison with the other groups. This shows that anti-inflammatory cytokines are not suppressed by BTx application.

Amylase and lipase are important digestive enzyme proteins and were suppressed by the application of BTx. This result could be related to why the suppression is prominent in AP but not significant in a normal pancreas. This is probably due to the disrupted ductal epithelium in AP. The application of BTx suppresses the over-activating receptors but does not disturb the normal activation of receptors.[23] This might also be due to the intrapancreatic nervous system. It is an advantage that BTx does not suppress the pancreas activity in normal conditions but suppresses the enzyme activity during AP. There have been some studies about suppression of chronic pancreatitis with celiac plexus block with BTx.[24]

Insulin and glucagon levels increase during AP.[25] Although serum levels of insulin increase during AP, the increase is not as high as expected for high glucose levels.[25] This is probably due to the release of stress hormones release, aside from the loss of pancreas cells to hypertrophy. In our study, glucagon levels were increased in AP, but there was no significant change in insulin levels. It was thought that the hyperglycemia during AP is dependent on hyperglucaconemia or related to stress.[26]

Insulin levels depend on several factors, such as amylin levels and insulin resistance.[26] The unchanged insulin levels might be due to the relation between amylin and cerulean, which is a CCK receptor agonist. Furthermore, insulin release is inhibited by pancreastatin, a 49-amino-acid peptide localized in pancreatic islet cells. This inhibitory effect is mediated by the presynaptic Ach release of the vagal innervation system.[27]

BTx may influence the insulin levels through inhibiting the release of pancreastatin, which will be the subject of future studies. The application of BTx did not decrease the levels of insulin and glucagon when compared to the AP group. This is probably related to alpha and beta-cell injury during AP. Another possible reason may be extrapancreatic glucagon secretion, which has been observed in pancreatectomized patients.[28] The glucose mechanism in AP is complex, and for this reason, further studies are needed to explain the action of BTx.

The pathological evaluation of AP showed severe centrilobular coagulation necrosis and severe inflammation, which are pathognomic signs of AP. The application of BTx to a normal pancreas only resulted in edema and mild inflammation around the pancreatic ducts. This is probably the result of mechanical injury due to the intraductal application. Pathological improvements after BTx application showed that the suppression of digestive enzymes protects the pancreas from the destructive effects of AP.

This is the first study to evaluate the effects of intraductal BTx application in AP, but it has some limitations. The AP model was created using cerulean, which is a CCK receptor agonist and might have a relationship with the cholinergic system. Although several studies agree about the action of CCK through the cholinergic system, the CCK receptor agonism is not clear. Thus, the future studies will be performed with other AP methods. Anti-secretory agents are the target for AP treatment by suppressing the pancreas. Bicarbonate secretion is important for protection against AP and is also abolished. Muscarinic receptor 1 (M1) is responsible for bicarbonate secretion. Therefore, telenzepine or pirenzepine usage may not be logical, but atropine, which is an M2 receptor blocker, could be a candidate for the future studies.

Conclusion

BTx inhibits the release of acetylcholine and might be a target for suppression of AP inflammation. Application of BTx through pancreatic duct in cerulean-induced AP can lead suppression of AP. Intraductal application of BTx showed histologic and biochemical improvement in AP without significant side effects.

Ethics Committee Approval: This study was approved by the Kocaeli University Animal Experiment Ethics Committee (Date: 03.09.2015, Decision No: KOU HADYEK 9/4-2015).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: T.U.Y., T.İ., F.C.E.; Design: T.U.Y., F.C.E., B.G.; Supervision: N.Z.U.; Resource: T.U.Y., N.Z.U., T.İ.; Materials: Ö.Y., E.A., B.G., H.Y.; Data: Ö.Y., E.A., B.G., H.Y.; Analysis: T.U.Y., F.C.E., B.G.; Literature search: Ö.Y., E.A., B.G., H.Y.; Writing: T.U.Y., Ö.Y., F.C.E.; Critical revision: T.İ., N.Z.U.

Conflict of Interest: None declared.

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DENEYSEL ÇALIŞMA - *ÖZ*

Deneysel akut pankreatit modelinde intraduktal botulinum toksin uygulaması enflamasyonu baskıladı

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AMAÇ: Akut pankreatit, pankreas enzim aktivitesinin arttığı pankreas enflamasyonudur. Pankreasın parasempatik uyarımı, pankreasın çeşitli fonksiyonlarında önemli rol oynar. Bu çalışmanın amacı, botulinum toksin'in (BTx), akut pankreatit'de pankreas aktivitesinin baskılayabileceğini göstermektir.
GEREÇ VE YÖNTEM: Sıçanlarda deneysel ön çalışmada 15 (u/kg) BTx, sıçan pankreasına direkt veya intraduktal uygulandı. Kan amilaz, lipaz, tripsinojen, insülin ve glukagon seviyeleri 10 gün sonra ölçüldü ve anlamlı fark saptanmadı. Çalışmaya klinik uygulanabilirlik açısından akut pankreatit modelinde intraduktal BTx uygulama ile devam edildi. Kontrol, akut pankreatit (AP), intraduktal BTx ve akut pankreatit'de BTX (AP+BTx) grupları sekizer sıçandan oluşturuldu. Akut pankreatiti intraperitoneal cerulein (20µg/kg) ile sağlandı. Sıçanlar 24 saat sonra serum amilaz, lipaz, IL-6, IL-1β, TNF-α, ve IL-10 ölçümü için sakrifiye edildi. Pankreas dokuları enflamasyon ve nekroz incelemesi için alındı.
BULGULAR: AP grubunda ortalama serum amilaz, lipaz, IL-6, IL-1β ve TNF-α değerleri diğer gruplara göre anlamlı yüksek bulundu. Kontrol, BTx, AP+BTx grupları arasında ortalama serum amilaz ve lipaz değerleri açısından anlamlı fark saptanmadı. AP grubunda ortalama serum insulin ve glukagon değerleri kontrol ve BTx grubuna göre anlamlı yüksek saptandı. Ortalama serum insülin ve glukagon düzeyleri AP ve AP+BTx grupları arasında anlamlı değildi. Patolojik incemelede AP+BTx grubunda AP'ye göre daha az sentrilobüler nekroz, inflamasyon izlendi.
TARTIŞMA: Intraduktal BTx uygulaması pankreas endojen aktivitesinde belirgin süpresyon yapmadan akut pankreatit'i baskılamıştır.

Anahtar sözcükler: Akut pankreatit; asetilkolin; botulinum toksin; pankreas kanalı; seruleim.

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