Effects of alpha-tocopherol on acute pancreatitis in rats

Halit Özgül, M.D.,1 Cihad Tatar, M.D.,1 Bahri Özer, M.D.,1 Hüsnü Aydin, M.D.,1 Soner Sari, M.D.,2 Songül Peltek Özer, M.D.3

1Department of General Surgery, Haseki Training and Research Hospital, İstanbul-Turkey
2Department of Biochemistry, Haseki Training and Research Hospital, İstanbul-Turkey
3Department of Pathology, Haseki Training and Research Hospital, İstanbul-Turkey

ABSTRACT

BACKGROUND: Acute pancreatitis is a disease with high morbidity and mortality, despite all the advances in technology. The overall mortality rate of acute pancreatitis is 10%, whereas the mortality rate in infected necrotizing pancreatitis is approximately 35%. In this study, we aimed to establish acute pancreatitis in rats in order to try out the alpha-tocopherol treatment protocol and to reveal the results biochemically and histopathologically.

METHODS: Twenty-four male Sprague–Dawley rats weighing between 300 and 350 g were used in the study. In Group 1, 80 µg/kg of normal saline was subcutaneously injected into eight rats; in Group 2, 80 µg/kg of cerulein was subcutaneously injected into eight rats; and in Group 3, 80 µg/kg of cerulein was subcutaneously injected into eight rats. In addition, 30 mg/kg of alpha-tocopherol was intraperitoneally injected into eight rats.

RESULTS: The mean Schoenberg score, serum amylase, and lipase and Neutrophil Gelatinase-Associated Lipocalin (NGAL) levels were statistically significantly higher in Group 2 than in Group 1. The mean Schoenberg score and serum amylase and lipase levels were statistically significantly lower in Group 3 than in Group 2.

CONCLUSION: In this experimental study rat model of cerulein-induced acute pancreatitis, 30 mg/kg of alpha-tocopherol was injected intraperitoneally to examine its effect on pancreatitis. The improvement was observed in the histopathological examination of pancreatic tissues. We think that alpha-tocopherol may have a therapeutic effect on pancreatic tissue.

Keywords: Alpha-tocopherol; pancreatitis; rat.

INTRODUCTION

Acute pancreatitis is an acute non-bacterial inflammation in which the pancreas is autodigested by its regurgitated own enzymes when they are in the active form and which can regress clinically and histologically.[1] Acute pancreatitis is a disease with high morbidity and mortality, despite all the advances in technology.[2] Acute pancreatitis may have a broad spectrum of pathologic findings, ranging from mild interstitial edema to severe hemorrhagic gangrene and necrosis. Similarly, clinical manifestations may occur in varying degrees, ranging from mild abdominal pain to hypotension, fluid sequestration, metabolic disturbances, and sepsis. The overall mortality rate of acute pancreatitis is 10%, whereas the mortality rate in infected necrotizing pancreatitis is approximately 35%.[3]

Respiratory complications are the leading cause of mortality in the short term in 95% of cases.[3]

In experimental pancreatitis models, studies are still being conducted to examine the efficacy of various therapeutic agents on treatment and to determine the parameters that can be used in measuring this efficacy. Cerulein is a cholecystokinin analog that leads to bile reflux into the pancreatic duct and thus pancreatitis by relaxing the sphincter of Oddi and stimulating the gallbladder contraction.[4] Cerulein has
been administered by the intravenous, subcutaneous, and intraperitoneal routes in experimental pancreatitis models.[5,6]

In this study, we aimed to establish acute pancreatitis in rats in order to try out the treatment protocol and to reveal the results biochemically and histopathologically.

MATERIALS AND METHODS

This experimental study was approved by the Ethics Committee of Bezmialem University Faculty of Medicine. It was carried out at the Experimental Animals Laboratory of Bezmialem University. Twenty-four male Sprague–Dawley rats weighing between 300 and 350 g (standard pelleted diet) were used in the study. Twenty-four rats were randomly divided into three groups of equal numbers.

**Group 1:** 80 µg/kg of normal saline (an equivalent volume of cerulein) was subcutaneously injected into eight rats 6 times at 1-hour intervals.

**Group 2:** 80 µg/kg of cerulein was subcutaneously injected into eight rats 6 times at 1-hour intervals.

**Group 3:** 80 µg/kg of cerulein was subcutaneously injected into eight rats 6 times at 1-hour intervals. In addition, 30 mg/kg of alpha-tocopherol was intraperitoneally injected into eight rats 2 times at 1-hour intervals.

Decapitation was performed 7 hours after the first injection of cerulein.

Biochemical Analysis

Approximately 7–8 cc of blood was obtained from each rat heart after decapitation and was kept at room temperature for 40 min. Then, the serum was separated by centrifugation at 3500 rpm at +4°C for 10 minutes. For amylase, lipase, Neutrophil Gelatinase-Associated Lipocalin (NGAL), myeloperoxidase (MPO), AST, and ALT, 0.5 cc of serum was placed into Eppendorf tubes to the biochemistry laboratory. It was measured according to the International Federation of Clinical Chemistry and Laboratory Medicine with the Roche Preanalytical Modular System device. The results of the groups were compared statistically to each other. A p-value <0.05 was considered statistically significant.

### Histopathological Examinations

After decapitation, the rats were laparotomized with a median incision. Pancreatic tissue was removed for histopathological evaluation. Specimens were fixed with a 10% formaldehyde solution. Pancreatic tissue sections were stained with the hematoxylin–eosin stain and examined under the light microscope. Edema, inflammation, vacuolization, and necrosis in pancreatic tissue were assessed using a score range of 0–4 (Schoenberg index) (Table 1). Moreover, pancreatic tissues of the groups with acute pancreatitis were stained with immunohistochemical stains. Myeloperoxidase staining demonstrated neutrophil infiltrates in pancreatic tissues. Neutrophil infiltration in pancreatic tissues was shown with the staining of IL-8 (also called neutrophil-activating peptide).

### Statistical Analysis

The SPSS 16.0 statistical software package (SPSS Inc., Chicago, USA) was used for statistical analysis. Categorical variables were expressed as number and percentage. Numerical variables were expressed as the mean and standard deviation. If numerical variables were normally distributed, the one-way analysis of variance was used to compare more than two independent groups. If numerical variables were not normally distributed, the Kruskal–Wallis test was used to compare more than two independent groups. Subgroup analyzes were performed by the Tukey test (parametric test) and the Mann–Whitney U test (non-parametric test), and they were interpreted by the Bonferroni correction. A p-value <0.05 was considered statistically significant.

### RESULTS

**Amylase**

The mean serum amylase level was 1537.00±123.26 in Group 1, 3316.13±432.49 in Group 2, and 2531.38±433.93 in Group 3. The mean serum amylase level was statistically significantly higher in Group 2 than in Group 1 (p<0.001). The mean serum amylase level was statistically significantly lower in Group 3 than in Group 2 (p<0.001) (Table 2).

<table>
<thead>
<tr>
<th>Table 1. Schoenberg’s scoring[10]</th>
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<tbody>
<tr>
<td><strong>Edema</strong></td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
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<td>4</td>
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</tbody>
</table>

* (microscopic field). 2–5 = mild pancreatitis. 5–8 = moderate pancreatitis. 8 and above = severe pancreatitis.
Lipase
The mean serum lipase level was 216.38±81.81 in Group 1, 3459.63±500.60 in Group 2, and 2450.38±808.69 in Group 3. The mean serum lipase level was statistically significantly higher in Group 2 than in Group 1 (p<0.0001). The mean serum lipase level was statistically significantly lower in Group 3 than in Group 2 (p=0.016) (Table 2).

NGAL
The mean serum NGAL level was 6.77±1.48 in Group 1, 14.43±5.13 in Group 2, and 10.37±2.38 in Group 3. The mean serum NGAL level was statistically significantly higher in Group 2 than in Group 1 (p=0.002). There was no statistically significant difference between Groups 2 and 3 in terms of the mean serum NGAL level (p=0.074) (Tables 2, 3).

MPO
The mean serum MPO level was 10.37±2.38 in Group 1, 14.39±1.77 in Group 2, and 14.90±1.92 in Group 3. There was no statistically significant difference between Groups 1 and 2 in terms of the mean serum MPO level. There was no statistically significant difference between Groups 2 and 3 in terms of the mean serum MPO level (p=0.088) (Tables 2, 3).

Histopathological Findings
In our study, pancreatitis was proven histologically in all 16 rats (Groups 2 and 3) in which it was planned to be established with subcutaneous injection of cerulein (80 µg/kg). Histopathological examinations revealed fat necrosis, interstitial edema, vacuolization, and polymorphonuclear cell infiltration in the groups with acute pancreatitis compared with the control group.

In our experimental rat model of cerulein-induced acute pancreatitis, neutrophil infiltration, vacuolization, fat necrosis, and edema in pancreatic tissue were evaluated by histopathological examination (Schoenberg index).

<table>
<thead>
<tr>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 1</th>
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<tbody>
<tr>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>196.63±162.96</td>
<td>155.88±29.42</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>75.50±14.73</td>
<td>78.13±10.59</td>
</tr>
<tr>
<td>Amylase</td>
<td>3316.13±432.49</td>
<td>2531.38±433.93</td>
</tr>
<tr>
<td>Lipase</td>
<td>3459.63±500.60</td>
<td>2450.38±808.69</td>
</tr>
<tr>
<td>NGAL</td>
<td>14.43±5.13</td>
<td>10.37±2.38</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>14.39±1.77</td>
<td>14.90±1.92</td>
</tr>
<tr>
<td>Pathology score</td>
<td>5.38±0.52</td>
<td>3.50±0.53</td>
</tr>
</tbody>
</table>

Table 3. Subgroup analyzes

| | Group 2 vs Group 3 | Group 1 vs Group 2 | Group 1 vs Group 3 |
| | p | p | p |
| Amylase (IU/mL) | 0.001 | <0.001 | <0.001 |
| Lipase | 0.016 | 0.001 | 0.001 |
| NGAL | 0.074 | 0.002 | 0.006 |
| Pathology score | 0.001 | 0.001 | 0.001 |

DISCUSSION
Pancreatitis is mainly caused by the auto-digestion of the pancreatic tissue resulting from the abnormal activation of pancreatic enzymes.[8,9] In the course of acute pancreatitis, systemic inflammatory conditions besides localized events occur. Oxygen free radicals play an important role in the pathogenesis of acute pancreatitis.[10]

Impaired microcirculation is also one of the important steps in the pathogenesis. There is an increase in the permeability of the endothelial layer of arterioles and venules. As a result, edema and microhemorrhages occur in the tissue due to extravasation of plasma and erythrocytes.[11] Willemer
et al. showed that interstitial edema occurred due to the PNL accumulation in pulmonary capillary vessels, damage to alveolar epithelial cells, and increased vascular permeability in cerulein-induced experimental acute pancreatitis model in rats. They also reported that these findings peaked at 12 hours and completely disappeared at 84 hours. For this reason, they emphasize the importance of early treatment. In many experimental studies, edematous pancreatitis has been established, and it has been aimed to prevent systemic complications.

Although different methods are used in experimental models of acute pancreatitis, an increase in pancreatic secretion is one of the most preferred methods for the pathophysiology of acute pancreatitis. Cerulein has been successfully used in many experimental studies with this effect. The effect of cerulein on the pancreas is dependent on the dose and duration. Cerulein dose and duration of administration are determined according to the form of pancreatitis to be created. Strowski et al. created an experimental model of acute edematous pancreatitis in rats where 10 µg/kg of cerulein was administered five times at 1-hour intervals. Konturek et al. created an experimental model of acute edematous pancreatitis in rats where 10 µg/kg of cerulein was administered subcutaneously.

The data obtained from many comprehensive experimental studies show that oxygen free radicals are produced as important mediators in the pathogenesis of many tissue injuries. Oxygen free radicals are involved in both the initial and later stages of the pathophysiology of acute pancreatitis. For this reason, experimental studies have been carried out to remove the efficacy of oxygen-free radicals in acute pancreatitis. In our study, 80 µg/kg of cerulein was subcutaneously injected into the rats six times at 1-hour intervals to create an experimental model of acute pancreatitis. Acute pancreatitis was created histopathologically and biochemically in the cerulein-treated groups (Groups 2 and 3). When these groups (Groups 2 and 3) were compared with the control group (Group 1), histopathological evaluations and biochemical measurements were found to be statistically significant (Table 3).

In experimental models of acute pancreatitis, serum amylase, and lipase levels were used as parameters to show the formation of pancreatitis. Serum amylase levels are elevated in acute pancreatitis, acute exacerbation of chronic pancreatitis, perforated and penetrating peptic ulcer, and pancreatic duct obstruction. The serum amylase level begins to rise within 3–6 hours in acute pancreatitis. Its urinary level begins to increase 6–10 hours after its serum level is increased. This alone has very little meaning. The amylase–creatinine clearance ratio (ACCR) is calculated. An ACCR greater than 5% suggests acute pancreatitis. We did not use this ratio because we also used lipase levels and histopathological evaluations in our study. Serum lipase levels are elevated in acute pancreatitis, perforated and penetrating peptic ulcer, and pancreatic duct obstruction. Lipase level can remain high until 14 days after the amylase level returns to normal.

While NGAL was initially characterized by its presence in neutrophil lysosomes, it was later observed that NGAL was expressed in various tissues, such as renal tubular epithelium, colon, prostate, and breast. Lipocalin (NGAL) is synthesized from the cells under stress. Infection, inflammation (synthesized from the secondary granules of active neutrophils), ischemia, and neoplastic transformation enhances NGAL expression. In our study, the mean serum NGAL level was statistically significantly higher in Group 2 than in Group 1, whereas there was no statistically significant difference between Groups 2 and 3 in terms of the mean serum NGAL level.

MPO is found in large quantities in neutrophils. This enzyme, stored in azurophilic granules of these cells, is responsible for killing of pathogenic microorganisms. These granulocytic cells engulf microorganisms via phagocytosis and break down and digest pathogens by producing reactive oxygen species, such as superoxide and hydrogen peroxide. In our study, there was no statistically significant difference between Groups 1 and 2 and between Groups 2 and 3 in terms of the mean serum MPO level (Table 3).

It has been reported that superoxide dismutase and catalase reduced the ultrastructural and biochemical injury associated with cerulein-induced acute pancreatitis in rats. According to the authors, active neutrophils secrete oxygen radicals and cause endothelial injury.

In a study by O’Donovan et al., conducted in an experimental rat model of cerulein-induced acute pancreatitis, endothelial cell injury, and edema formation were detected in pancreatic tissue, and this event was thought to be related to neutrophils. In experimental pancreatitis models, acute pancreatitis showed the same morphological characteristics with that in humans. A clinical condition was also established to be biochemically similar to that in humans by increased serum amylase and lipase levels. It was also seen that non-fatal pulmonary injury occurred in acute pancreatitis and was similar to that in humans.

Alpha-tocopherol has a protective effect on membrane stabilization and an inhibitory effect on lipid peroxidation. It is also known as an antioxidant. It easily wedges between the membrane phospholipids due to its high lipid solubility and reduces unsaturated fatty acids with 20 carbon atoms. Thus, it prevents lipid peroxidation in biomembranes caused by oxygen free radicals. Moreover, alpha-tocopherol is converted into alpha-tocopheryl quinone by combining with oxygen free radicals.
radicals, and thus it undertakes the free radical scavenging process.

Jiang et al. showed that alpha-tocopherol and tocotrienol-rich fraction reduce oxidative stress, ameliorate inflammation and fibrosis, and down-regulate the mRNA expression of TGF-β1 and collagen-α1 in chronic pancreatitis.

Li et al. investigated the effects of alpha-tocopherol on pancreatic fibrosis and survival in rats with experimental chronic pancreatitis. They reported that alpha-tocopherol treatment elevates the survival rate, extenuates fibrosis, and increases relative pancreatic weight in the chronic pancreatitis model.

It has been reported that the oral administration of alpha-tocopherol significantly ameliorated the symptoms of diabetic nephropathy.

Previous studies have highlighted the potential of using alpha-tocopherol for cancer chemoprevention. However, several studies indicated that alpha-tocopherol might be ineffective in the prevention of prostate cancer, lung cancer, and breast cancer.

In our experimental rat model of cerulein-induced acute pancreatitis, 30 mg/kg of alpha-tocopherol was injected intraperitoneally at 1-hour intervals 2 hours after the first injection of cerulein to examine its effect on pancreatitis. While the serum amylase, lipase, and NGAL levels and the mean Schoenberg score were statistically significantly higher in Group 2 than in Group 1, the serum amylase and lipase levels and the mean Schoenberg score were statistically significantly lower in Group 3 than in Group 2.

In this experimental study, we aimed to establish an acute pancreatitis model in rats, to reveal the natural process of acute pancreatitis, and to examine the efficacy of the agent which can be tried in treatment. We observed that acute pancreatitis developed as both histopathological and laboratory findings in rats after subcutaneous injection of cerulein. In an experimental model of acute pancreatitis, it was seen that the serum lipase, amylase, and NGAL levels increased significantly statistically, the serum MPO level did not increase significantly statistically, and the serum amylase and lipase levels decreased significantly statistically in the rats given alpha-tocopherol. In addition, the improvement was observed during the histopathological examination of pancreatic tissues. These findings suggest that alpha-tocopherol may have a positive effect on treatment.

Conclusion

In conclusion, we think that alpha-tocopherol may have a therapeutic effect on pancreatic tissue. However, new studies need to be conducted with this regard.

Conflict of interest: None declared.

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DENEYSEL ÇALIŞMA - ÖZET

Şıçanlarda alfa tokoferol'un akut pankreatit üzerine etkileri

Dr. Halit Özgül,¹ Dr. Cihad Tatar,¹ Dr. Bahri Özer,¹ Dr. Hüsnü Aydin,¹ Dr. Soner San,2 Dr. Songül Peltek Özer³

¹Haseki Eğitim ve Araştırma Hastanesi, Genel Cerrahi Kliniği, İstanbul
²Haseki Eğitim ve Araştırma Hastanesi, Biyokimya Kliniği, İstanbul
³Haseki Eğitim ve Araştırma Hastanesi, Patoloji Kliniği, İstanbul

AMAÇ: Akut pankreatit, teknolojideki tüm gelişmelerle rağmen morbidity ve mortalitesi yüksektir. Akut pankreatitte mortalite oranı tüm hastalar için %10 ile, bu oran enfekte olmuş nekrotizan pankreatitli hastalarda yaklaşık olarak %35'tir. Biz, bu çalışmada öncelikle sıçanlarda akut pankreatit oluşturmayı, alfa tokoferol tedavi protokolünü denemeyi ve sonuçları biyokimyasal ve histopatolojik olarak ortaya koymayı amaçladık.

GEREÇ VE YÖNTEM: Çalışmada 24 adet erkek sıçan kullanıldı. Grup 1: Kontrol grubu olaraksekiz sıçana birer saatlik arayla altı kez toplam 80 mikrogram/kg (cerulein ile eş hacimli) serum fizyolojik subkutan enjeksiyon edildi. Grup 2: Sekiz sıçana birer saat saata birer saat toplam 80 ug/kg cerulein subkutan enjeksiyon edildi. Grup 3: Sekiz sıçana birer saat saata iki toplam 80 mikrogram/kg cerulein ve birer saat saata iki kez 30 mg/kg alfa-tokoferol intraperitoneal enjeksiyon edildi.

BULGULAR: Ortalama Schoenberg skoru, serum amilaz, lipaz ve NGAL seviyeleri Grup 2'de Grup 1'e göre istatistiksel olarak anlamlı derecede yüksekti. Ortalama Schoenberg skoru, serum amilaz ve lipaz seviyeleri Grup 3 de Grup 2’ye göre istatistiksel olarak anlamlı derecede düşüktü. TARTIŞMA: Cerulein ile oluşturulmuş pankreatit modelinde pankreatit üzerine olan etkisini incelemek amacıyla 30 mg/kg alfa-tokoferol intraperitoneal olarak uygulandı. Alfa-tokoferolün pankrez dokusu üzerinde tedavi edici etkiyi olayla ve belirli hücrelerin düzümmekteyiz.

Anahtar sözcükler: Alfa tokoferol; pankreatit; sıçan.


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6