Protective effects of etanercept and methylprednisolone on pancreatic damage in cerulein-induced acute pancreatitis

Erol Kilic, MD, *Ramazan Amanvermez*, PhD, *Mehmet Kefeli*, MD, *Cafer Polat*, MD, *Murat Gunay*, MD.

ABSTRACT

الأهداف: فحص التأثير الدوائي لعقار الإثانيرسيب، و عقار ميثيل بريدنيزونون (MP) في التهاب البنكرياس الحاد (AP) نتيجة استخدام السيرولين في نموذج التجربة للجرذ.

الطريقة: أجريت دراسة الحالة في مركز أبحاث التجارب – جامعة اندوكوز مايس – سامسن – تركيا خلال الفترة من ديسمبر 2008م حتى أكتوبر 2009م. قمنا بتقسيم 40 فأر من نوع سبراغ داولي إلى 5 مجموعات (العدد=8\لكل مجموعة)، المجموعة الأولى 1 أجريت لها عملية خادعة، و المجموعة الثانية 2 المصابة بالتهاب البنكرياس و استخدم فيها السيرولين لمدة 20 ساعة، المجموعة الثالثة 3 أعطيت عقار الإثانيرسيب بمقدار 5mg/kg، المجموعة الرابعة 4 أعطيت عقار ميثيل بريدنيزونون 10mg/kg، و المجموعة الخامسة 5 استخدمت عقار الإثانيرسيب، و عقار ميثيل بريدنيزونونMP. تم حقن الفئران في المجموعة 3، و 4، و 5 باستخدام السيرولين لتحريض التهاب البنكرياس في 20 ساعة. بعد العلاج، تم أخذ البنكرياس و الدم للتحليل الكيميائي و النسيجي.

النتائج: تطور التهاب البنكرياس في أنسجة جميع الفئران المعالجة باستخدام السيرولين بعد 20 ساعة. انخفضت نتائج أنسجة التهاب البنكرياس و مستويات المصل للإنزيم في المجموعة 5 مقارنة مع المجموعة 2. انخفض التهاب البنكرياس و النقاط النسيجية الكلية بشكل مهم إحصائياً في أنسجة البنكرياس في الساعة 20 بعد العلاج باستخدام عقار الإثانيرسيب و عقار ميثيل بريدنيزونون MP في المجموعة 5، مقارنة بالمجموعة 2، و 3، و4.

خاتمة: يضعف عقار الإثانيرسيب و عقار ميثيل بريدنيزونون MP التهاب البنكرياس AP و يحدث الضرر بشكل مهم في الفئران وذلك في المراحل الأولية لالتهاب البنكرياس نتيجة استخدام السيرولين.

Objectives: To examine the pharmacological effect of etanercept and methylprednisolone (MP) on acute pancreatitis (AP) induced by cerulein in an experimental rat model.

Methods: The present study was carried out in the Experimental Research Center, Ondokuz Mayis University, Samsun, Turkey between December 2008 and October 2009. Forty adult Sprague-Dawley rats were divided into 5 groups (n=8): 1 - sham, 2 - cerulein induced pancreatitis (over 20 hours), 3 - etanercept (5 mg/kg, intraperitoneal), 4 - MP (10 mg/kg, intramuscular), 5 - etanercept plus MP. The rats in groups 3, 4, and 5 were cerulein-induced pancreatitis at 20 hours, as well. After the treatment, the pancreas and blood were taken for histopathological and biochemical analysis.

Results: All cerulein-treated rats developed biochemical and histopathological AP after 20 hours. Histological findings of pancreatitis and serum levels of amylase and lipase were lower in group 5 compared to group 2. Pancreatic inflammation and total pathological score were statistically reduced in the tissues of the pancreas at 20 hours after the treatment of etanercept plus MP in group 5 compared to groups 2, 3, and 4.

Conclusion: In the early stage of cerulein induced AP, the administration of etanercept plus MP attenuated pancreatic inflammation and significant damage in rats.

Acute pancreatitis (AP) is an inflammatory condition of the pancreatic tissue with a high mortality rate (approximately 10%). Biliary tract stones, alcohol abuse, hyperlipidemia, hypercalcemia, post-endoscopic retrograde cholangiopancreatography (ERCP), and

intoxication are common causes of AP. This disease may cause life-threatening events resulting in different organ failure as well.[1-3] It is characterized by local pancreatic inflammation with recruitment of leukocytes. Evidence from experimental models[4-7] suggests that premature activation of trypsinogen represents a critical initiating event that leads to acinar cell damage. The activated pancreatic digestive enzymes contribute at an early stage to injury of acinar cells, and consequently to the inflammation of the pancreas. In this condition, tissue, and cell membranes break down causing edema, vascular damage, hemorrhage, and necrosis.[4] Meanwhile, the acinar cell damage results in local activation of the immune system, including macrophages, fibroblasts, T cells, and endothelial cells among others.[5,6] Once the disease process is initiated, common inflammatory and repair pathways may also be provoked. The disease can progress in 3 phases: local inflammation of the pancreas, a generalized inflammatory response, and the final stage of multiorgan dysfunction. Clinically, it may be assumed that in some patients, clinical and histological findings of pancreatic local inflammation can be resolved with supportive treatment. However, the disease may progress to systemic illness for some unfortunate patients. Uncontrolled local inflammation leads to systemic inflammatory response syndrome, and it is believed that this systemic response is ultimately responsible for most of the morbidity and mortality.[6-9] In recent reports,[9] inflammatory factors seem to play a major role in the pathogenesis of pancreatitis. Pro-inflammatory cytokines (such as interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF)-α) act as modulators directly correlated to most pancreatitis-associated mortality.[9] In particular, TNF-α overproduction occurs from the early stages of AP, and is pivotal in the induction of inflammatory genes, cell death, endothelial up-regulation, and in the recruitment and activation of immune cells.[6-10] There is no specific treatment for AP, and treatment remains mostly supportive, based on etiology. Effective therapeutic agents are required to reduce morbidity and mortality rates of acute pancreatitis-associated life loss. Etanercept, which is a soluble TNF-α binding agent, was shown to diminish the development of cerulein-induced AP, but it is ineffective in the prevention of mild to moderate post-ERCP pancreatitis, as stated in the literature.[3,11] Like most corticosteroids, methylprednisolone (MP) is typically used for its anti-inflammatory effects. However, the therapeutic role of MP in AP is less known. The function of etanercept plus MP is unclear as a therapeutic agent in the prevention, and treatment of AP.[2] The aim of the present study was to investigate whether etanercept, MP, and etanercept plus MP can prevent, or diminish pancreatic damage in cerulein-induced AP, as defined by enzyme elevations and histopathological findings.

Methods. *Animals.* Forty adult Sprague-Dawley rats (age: 7-8 months) weighing 250-270 g were used for this experimental research. The rats were kept in cages with wood chip bedding, and fed on standard laboratory chow and water ad-libitum. They were maintained on a 12 hour light/dark cycle, with a constant room temperature at 22 ± 1^0C. The local ethical committee of Ondokuz Mayis University approved all animal procedures, and the experimental protocol. This study was carried out in the Experimental Research Center, Ondokuz Mayis University, Samsun, Turkey between December 2008 and October 2009.

Animal treatment. Rats were randomly allocated into the following groups (n=8): 1. sham-treated group in which identical treatments to group 2 were performed, except that the saline was administered instead of cerulein (intraperitoneally [i.p.]); 2. cerulein-induced pancreatitis group (at 20 hours): rats were treated hourly (5 times) with cerulein (total 80 µg/kg, i.p., suspended in saline solution), and an additional 15 hours was allowed to pass for a complete pancreatitis induction. 3. etanercept group, except for the administration of etanercept (5 mg/kg, i.p., dissolved in saline solution), that was given hourly (2 times) at the end of the fifth hour after the last cerulein application; 4. MP group, the administration of MP (10 mg/kg, intramuscular [i.m.]) which was given hourly (2 times) at the end of the fifth hour after the last cerulein application; 5. etanercept plus MP group, the administration of MP (10 mg/kg, i.m.) along with etanercept (5 mg/kg, i.p.) that was given hourly (2 times) at the end of the fifth hour after the last cerulein application. At the end of the experiments, the rats were anesthetized by intramuscular injection of ketamine. After blood samples were obtained by direct intracardiac puncture with an injector, all rats were sacrificed by exsanguinations under anesthesia at 20 hours after the induction of pancreatitis. The rats in group one were killed at 20 hours. The pancreas was resected with midline laparotomy under sterile conditions, and this organ was fixed in 10% buffered formaldehyde for histopathological examination. Serum pancreatic amylase, lipase, and lactate dehydrogenase (LDH) levels were measured by a clinical chemistry laboratory at 20 hours.

Histopathological study. For light microscopy, part of the pancreas was fixed with 24 hours buffered neutral formalin, and processed for routine tissue follow-up with Shandon Citadel 2000 apparatus (Thermo Fisher Scientific Inc., Waltham, Maryland, USA). The tissues were embedded in paraffin blocks, and then cut at 4-6 µm thick for histologic evaluation. The histologic

section was stained with hematoxylin and eosin. The stained sections were evaluated by a pathologist who was uninformed of the groups. The histopathologic findings such as edema, inflammation, vacuolization, and necrosis in the pancreas were evaluated. Each histopathologic finding was scored from 1-4 according to the criteria defined by Rongione et al.[12] Edema was scored semi-quantitatively: score 0 - no tissue edema; scores 1 (+), 2 (++), 3 (+++) - for diffuse expansion of interlobar septa. Inflammation was scored with respect to the severity of inflammatory cell infiltration in the parenchyma: score 0 - no inflammation; 1 - only around the ductus; 2 - <50% of lobules; 3 - 51 to 75% of lobules, 4 - >75% of lobules. Necrosis was scored according to necrotic cells count at a high power field (HPF) with the following scale: score 0. no necrosis; 1. one-4 necrotic cells/HPF; 2. 5-10 necrotic cells/HPF; 3. 11-16 necrotic cells/HPF; 4. >16 necrotic cells/HPF. Vacuolization was scored semi-quantitatively: score 0 - no vacuolization; 1 - periductal; 2 - focal; 3 - diffuse; 4 - severe.

Statistical analysis was performed using the Statistical Package for Social Sciences version 15.0 software for Windows (SPSS Inc., Chicago, IL., USA). The Kruskall-Wallis analysis of variances was used to compare the whole groups, and then the pairwise comparisons were carried out by Mann-Whitney U test with Bonferroni correction. Biochemical parameters were normally distributed, so the statistical evaluations were carried out by one-way analysis of variance, and then the pairwise comparisons were carried out by Tukey multiple comparison test. A *p*-value of <0.05 was considered to be statistically significant.

Results. *Biochemical parameters.* Serum levels of pancreatic amylase and lipase were significantly higher in group 2 than in group 1 (*p*=0.001). Both enzyme values were decreased in group 3 when compared to group 2. In addition, their levels were much lower in group 5 compared to group 2 as shown in **Table 1**.

No elevation in the serum values of pancreatic amylase and lipase, and histopathological alterations was observed in the pancreatic tissue from group 1 (**Figure 1a, Table 2**), excluding the least of edema compared to the other groups. Histopathological examination of pancreas sections after cerulein administration revealed tissue damage characterized by edema, vacuolization, inflammation, and cell necrosis (**Figure 1b, Table 2**).

Effect of etanercept on pancreatic damage during the experimental AP. In the pancreas tissue, sections obtained from group 3 rats, 20 hours after cerulein injection, pathologist of the study indicated that there was less tissue damage in group 3 (**Figure 1c**). Additionally, the total pathological score was significantly lower in group 3 than in group 2 (**Table 2**) (*p*=0.01).

Effect of MP on pancreatic damage during experimental AP. Evidence of AP is the accumulation of neutrophils in the pancreas, which augment the

Table 1 - Serum levels of pancreatic amylase, lipase, and LDH in the experimental groups (n=8/group).

Groups	Pancreatic amylase (U/L)	Lipase (U/L)	LDH (IU/L)
1	1291.3 ± 217.4	7.5 ± 0.9	474.3 ± 156
2	5172 ± 2557	110.1 ± 87	2319.6 ± 831
3	3085 ± 1228	54 ± 46	1725.6 ± 880
4	4243 ± 2341	87.3 ± 79	1910.3 ± 776
5	3836 ± 1399	28.8 ± 19	2732.6 ± 440

Data are expressed as mean ± standard deviation.
LDH - lactate dehydrogenase

Table 2 - Effectiveness of etanercept-methylprednisone treatment, and pancreatic damage according to pathological findings.

Groups	n	Pathological findings				Total score
		Edema	Inflammation	Vacuolization	Necrosis	
1	8	0.25 ± 0.46 0.0 (0;1)	0.0 ± 0.0 0.0 (0;0)	0.0 ± 0.0 0.0 (0;0)	0.0 ± 0.0 0.0 (0;0)	**0.25 ± 0.46** **0.0 (0;1)**
2	8	0.50 ± 0.53[a] 0.5 (0;1)	2.14 ± 0.83[b] 2.0 (1;3)	1.25 ± 0.46[c] 1.0 (1;2)	1.25 ± 0.70[d] 1.0 (0;2)	**5.12 ± 1.55** **5.0 (3;8)**
3	8	0.13 ± 0.35 0.0 (0;1)	1.63 ± 0.74 1.50 (1;3)	0.63 ± 0.74 0.50 (0;2)	0.13 ± 0.35 0.0 (0;1)	**2.50 ± 1.41[e]** **2.0 (1;5)**
4	7	0.14 ± 0.37 0.0 (0;1)	1.14 ± 0.90 1.0 (0;2)	0.57 ± 0.53 1.0 (0;1)	0.14 ± 0.37 0.0 (0;1)	**2.00 ± 1.41[f]** **3.0 (0;3)**
5	8	0.0 ± 0.0 0.0 (0;0)	0.75 ± 0.46 1.0 (0;1)	0.13 ± 0.35 0.0 (0;1)	0.0 ± 0.0 0.0 (0;0)	**0.87 ± 0.64[g]** **1.0 (0;2)**

Data are expressed as mean ± standard deviation, and median (minimum;maximum) under each pathological finding. Statistical differences are indicated as follows: [a]*p*=0.001 versus group 5 for edema; [b]*p*=0.01 versus group 5 for inflammation; [c]*p*=0.01 versus group 5 for vacuolization; [d]*p*=0.001 versus group 5 for necrosis; [e]*p*=0.01 versus group 2, [f]*p*=0.01 versus group 2, [g]*p*=0.001 versus group 2 for total score
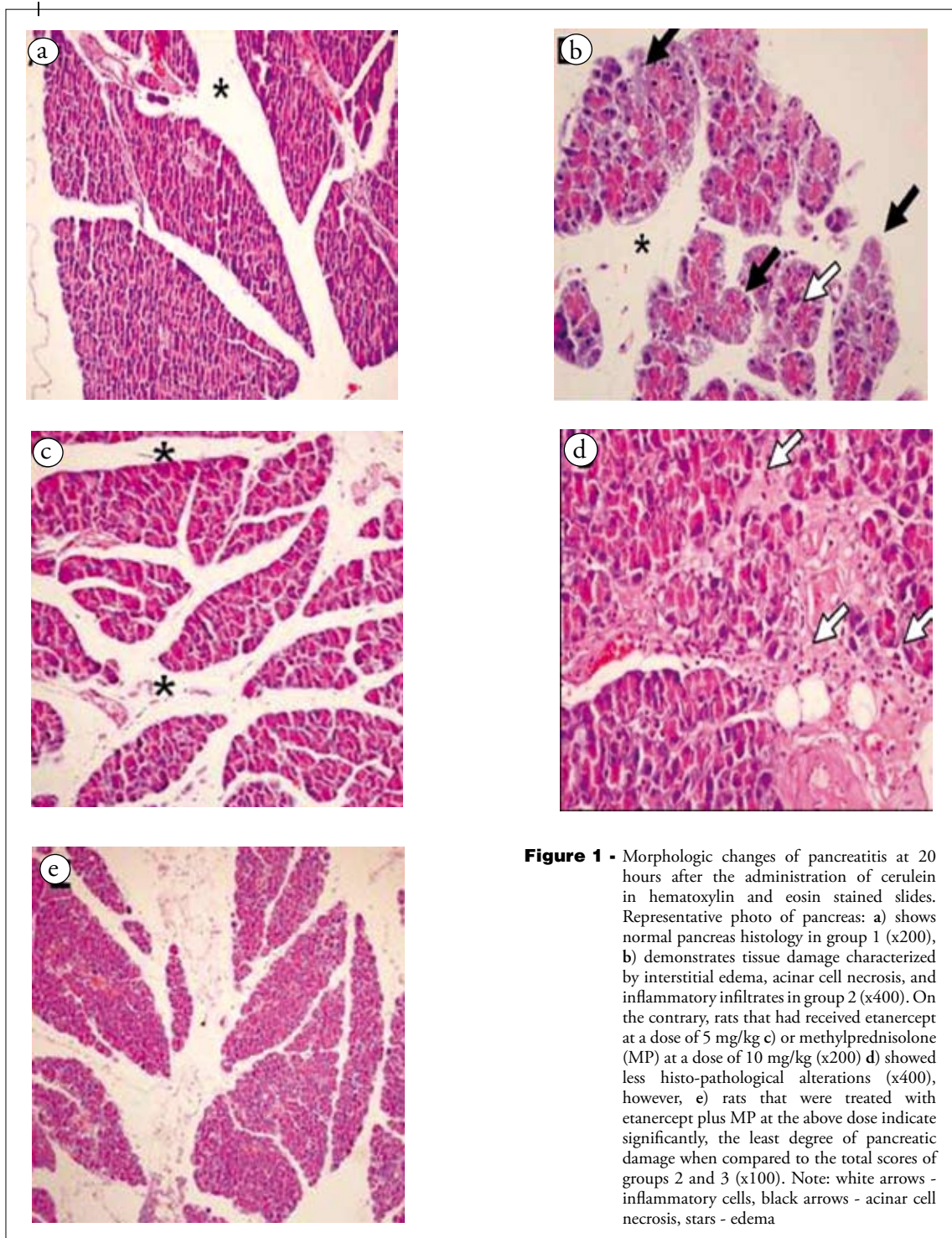
Figure 1 - Morphologic changes of pancreatitis at 20 hours after the administration of cerulein in hematoxylin and eosin stained slides. Representative photo of pancreas: **a**) shows normal pancreas histology in group 1 (x200), **b**) demonstrates tissue damage characterized by interstitial edema, acinar cell necrosis, and inflammatory infiltrates in group 2 (x400). On the contrary, rats that had received etanercept at a dose of 5 mg/kg **c**) or methylprednisolone (MP) at a dose of 10 mg/kg (x200) **d**) showed less histo-pathological alterations (x400), however, **e**) rats that were treated with etanercept plus MP at the above dose indicate significantly, the least degree of pancreatic damage when compared to the total scores of groups 2 and 3 (x100). Note: white arrows - inflammatory cells, black arrows - acinar cell necrosis, stars - edema

tissue damage. Their infiltration into inflamed tissue contributes to the inactivation of foreign antigens, and to remodeling of the injured tissue, but an exaggerated recruitment into the pancreas accounts for tissue destruction via the production of reactive oxygen species, granule enzymes, and cytokines that further amplify the inflammatory response.[13] Therefore, we determined a higher inflammation, necrosis, and total score in group 2 compared to group one. However, the treatment of MP during rat AP statistically led to lower inflammation, necrosis, and total pathological score in group 4 compared to group 2 (p=0.01) (**Figure 1d, Table 2**).

Effects of etanercept plus MP on pancreatic damage during experimental AP. The levels of pancreatic amylase and lipase were found to be lower in the blood of rats in group 5 with the application of etanercept plus MP than those of group 2 (**Table 1**). In addition, total histopathological score was found significantly lower in group 5 (**Figure 1e, Table 2**) (p=0.001). In the administration of etanercept plus MP, it is important to note that the total pathological score was considerably near to normal with respect to group 1.

Discussion. The AP is one of the common causes of an acute abdomen in clinic with a rapid onset and dangerous pathological condition. Its incidence has been elevating over recent years.[1,6] However, evidence-based pharmacological approaches are limited in the treatment of AP, and none of the therapeutic agents used for therapy are effectively curative.[2]

The TNF-α is a key pro-inflammatory cytokine that causes vasodilation, enhances microvascular permeability, activates leukocytes, and induces the release of other cytokines and the expression of cellular adhesion molecules, which propagate the inflammatory cascade. It induces acute inflammation and release superoxide anion.[4,5] It plays an integral role in the development of cerulein-pancreatitis,[3] and a role in the pathogenesis of AP.[14,15] In previous studies, different anti-TNF-α strategies have been evaluated in experimental pancreatitis. Inhibition of TNF-α attenuated the disease' severity, and improved survival in experimental models of AP.[16,17] In addition to these reports, Oruc et al[18] indicated that Infliximab (a monoclonal anti-TNF-α antibody) diminishes the course of edematous and necrotizing pancreatitis in rats. As cited previously in the text, etanercept, a novel anti-TNF-α agent, which binds specifically to TNF and blocks its interaction with cell surface TNF receptors, reduced the development of AP in mice. But, it is ineffective in the prevention of mild to moderate post-ERCP pancreatitis in canines. These animal model studies are interested in the prevention of the development of AP. Our study is different from the last 2 reports, as we planned to treat with etanercept in the early stages of AP and during the course of the disease, and etanercept (5 mg/kg, i.p.) was given hourly (2 times) at the end of the fifth hour after the last cerulein injection. In the former study, etanercept was administered at one hour after the first cerulein injection (5 or 10 mg/kg), and in the latter study, etanercept was administered one day prior to post-ERCP pancreatitis (1 mg/kg, subcutaneous). Malleo et al[3] and Granell et al[19] indicated that during the early stages of pancreatitis, TNF-α and its soluble receptors are released to systemic circulation and maintained at high concentrations. The TNF-α reduction by etanercept may slow pancreatic

inflammation and damage in AP. As the plasma levels of TNF-α reported by Malleo et al[3] were significantly reduced in etanercept-treated animals with AP after 24 hours, our findings is in agreement with this study that etanercept has a protective effect on pancreatic damage in the course of AP at 20, or 24 hours.

Corticosteroids are widely used for the suppression of inflammatory responses.[20] Currently these drugs are being used in a wide variety of inflammatory diseases affecting many organs, such as rheumatoid arthritis, acute gouty arthritis, and systemic lupus erythematosus. For instance, MP, which is a synthetic glucocorticoid drug can be used to achieve prompt suppression of inflammation.[21,22] The MP (5-40 mg/kg, 6-72 hours) pre-treatment reduced the serum amylase activity, the pancreatic levels of TNF-α and IL-6, the pancreatic myeloperoxidase activity, and morphological parameters of the disease during cholecystokinin octapeptide-induced AP in rats.[21] Dexamethasone (0.5 mg/kg) administered after induction of AP ameliorated the inflammatory mediators, pancreatic damage, and then improved the survival rate of rats with AP.[22] Kimura et al[23] reported that glucocorticoids attenuated pancreatic damage by protecting acinar cells during cerulein-induced AP. In our study, MP at the dose of 10 mg/kg in cerulein-induced AP led to decreased serum pancreatic amylase (17.9%), and lipase (20.7%) levels in group 4 compared to group 2 (**Table 1**). In addition, pancreatic inflammation was suppressed by 46.7%, and pancreatic necrosis was reduced by 88.8%, and also total pancreatic pathologic score was significantly attenuated by 60.9% in pancreas tissues from the rats in group 4 compared to group 2. As a result of these findings, MP has a protective effect against damage in AP. Similarly, Takaoka et al[24] suggested that the beneficial effects of MP arose both from the inhibition of the local inflammation in the pancreas, and the blockade of generalized inflammation through the activation of a cytokine cascade, and hemodynamic disturbance.

In the present study, we hypothesized whether treatment with etanercept plus MP could be given in the early stages of pancreatitis, the long-term effects, or the pancreatic pathological features during of the disease might be diminished or prevented. In our study, pancreatic inflammation was ameliorated by 64.9% in group 5 compared to group 2. Pancreatic amylase and lipase values decreased in the blood 20 hours after the treatment of these drugs. Moreover, this combined treatment prevented pancreatic necrosis and edema formation, as these findings did not appear in the pancreatic tissues from the rats in group 5 with AP after the application of these 2 drugs. It is important to note that pancreatic summary score was statistically reduced by 83% in this group. Furthermore, the summary score was considerably near to normal with respect to group

one as shown in **Table 2** and **Figure 1e**. Inflammatory cells in particular, neutrophils, which play a crucial role in the development and full manifestation of AP are very active in this disease.[25] Etanercept plus MP therapy in inflamed pancreatic tissue may result in a significant reduction in inflammatory activity.

In conclusion, these findings suggest that treatment with etanercept and MP diminishes pancreatic damage during the early stages of cerulein-induced AP in rats. The pharmacological effect of MP in pancreatic inflammation and total tissue damage seems to be better than etanercept, and the combination of these drugs makes the best of pharmacological therapy in this study. Simultaneous usage of these drugs may be a novel target by therapeutic applications for treating pancreatic inflammation. However, there are deficiencies of research materials such as, low number of experimental animals, the values of IL-1α and TNF-α not measured in the pancreas or blood in this study. Therefore, studies concerning the use of etanercept plus MP in the treatment of AP should be undertaken in the future.

References

1. Steer ML. Exocrine pancreas. In: Tavsend CM, Beauchamp RD, Evers BM, Mattox KL, editors. Sabiston Textbook of Surgery: The Biological Basis of Modern Surgical Practice. 18th ed. Philadelphia (PA): Saunders, Elsevier Inc.; 2008: p. 1589-1623.
2. Bang UC, Semb S, Nojgaard C, Bendtsen F. Pharmacological approach to acute pancreatitis. *World J Gastroenterol* 2008; 14: 2968-2976.
3. Malleo G, Mazzon E, Genovese T, Di Paola R, Muià C, Centorrino T, et al. Etanercept attenuates the development of cerulein-induced acute pancreatitis in mice: a comparison with TNF-alpha genetic deletion. *Shock* 2007; 27: 542-551.
4. Pereda J, Sabater L, Aparisi L, Escobar J, Sandoval J, Vina J, et al. Interaction between cytokines and oxidative stress in acute pancreatitis. *Curr Med Chem* 2006; 13: 2775-2787.
5. Halangk W, Lerch MM. Early events in acute pancreatitis. *Gastroenterol Clin North Am* 2004; 33: 717-731.
6. Bhatia M, Wong FL, Cao FL, Lau HY, Huang JL, Puneet P, et al. Pathophysiology of acute pancreatitis. *Pancreatology* 2005; 5: 132-144.
7. Raraty MG, Connor S, Criddle DN, Sutton R, Neoptolemas JP. Acute pancreatitis and organ failure: pathophysiology, natural history, and management strategies. *Curr Gastroenterol Rep* 2004; 6: 99-103.
8. Bauman B, Wagner M, Aleksic T, Wichert G, Weber CK, Adler G, et al. Constitutive IKK2 activation in acinar cells is sufficient to induce pancreatitis in vivo. *J Clin Invest* 2007; 117: 1502-1513.
9. Hirota M, Nozawa F, Okabe A, Shibata M, Beppu T, Shimada S, et al. Relationship between plasma cytokine concentration and multiple organ failure in patients with acute pancreatitis. *Pancreas* 2000; 21: 141-146.
10. Granger J, Remick D. Acute pancreatitis: models, markers, and mediators. *Shock* 2005; 24: 45-51.
11. Buscaglia JM, Simons BW, Prosser BJ, Ruben DS, Giday SA, Magno P, et al. Etanercept, a TNF-alpha binding agent, is ineffective in the prevention of post-ERCP pancreatitis in canines. *JOP* 2008; 9: 456-467.
12. Rongione AJ, Kusske AM, Kwan K, Ashley SW, Reber HA, McFadden DW. Interleukin-10 reduces the severity of acute pancreatitis in rats. *Gastroenterology* 1997; 112: 960-967.
13. Cuzzocrea S, Genovese T, Mazzon E, Di Paola R, Muia C, Britti D, et al. Reduction in the development of cerulein-induced acute pancreatitis by treatment with M40401, a new selective superoxide dismutase mimetics. *Shock* 2004; 22: 254-261.
14. Yadav D, Agarwal N, Pitchumoni CS. A critical evaluation of laboratory tests in acute pancreatitis. *Am J Gastroenterol* 2002; 97: 1309-1318.
15. Norman JG, Fink GW, Franz MG. Acute pancreatitis induces intrapancreatic tumor necrosis factor gene expression. *Arch Surg* 1995; 130: 966-970.
16. Denham W, Fink G, Yang J, Ulrich P, Tracey K, Norman J. Small molecule inhibition of tumor necrosis factor gene processing during acute pancreatitis prevents cytokine cascade progression and attenuates pancreatitis severity. *Am Surg* 1997; 63: 1045-1049.
17. Hughes CB, Gaber LW, Mohey el-Din AB, Grewal HP, Kotb M, Mann L, et al. Inhibition of TNF alpha improves survival in an experimental model of acute pancreatitis. *Am Surg* 1996; 62: 8-13.
18. Oruc N, Ozutemiz O, Yukelsen V, Nart D, Celik HA, Yuce G, et al. Infliximab: a new therapeutic agent in acute pancreatitis? *Pancreas* 2004; 28: 1-8.
19. Granell S, Pereda J, Gomez-Cambronero L, Cassinello N, Sabater L, Closa D, et al. Circulating TNF-alpha and its soluble receptors during experimental acute pancreatitis. *Cytokine* 2004; 25: 187-191.
20. Barnes PJ. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci (Lond)* 1998; 94: 557-572.
21. Rakonczay Z Jr, Duda E, Kaszaki J, Iványi B, Boros I, Lonovics J, et al. The anti-inflammatory effect of methylprednisolone occurs down-stream of nuclear factor-kappaB DNA binding in acute pancreatitis. *Eur J Pharmacol* 2003; 464: 217-227.
22. Wang ZF, Pan CE, Lu Y, Liu SG, Zhang GJ, Zhang XB. The role of inflammatory mediators in severe acute pancreatitis and regulation of glucocorticoids. *Hepatobiliary Pancreat Dis Int* 2003; 2: 458-462.
23. Kimura K, Shimosegawa T, Sasano H, Abe R, Satoh A, Masamune A, et al. Endogenous glucocorticoids decrease the acinar cell sensitivity to apoptosis during cerulein pancreatitis in rats. *Gastroenterology* 1998; 114: 372-381.
24. Takaoka K, Kataoka K, Sakagami J. The effect of steroid pulse therapy in the development of acute pancreatitis induced by closed duodenal loop in rats. *J Gastroenterol* 2002; 37: 537-542.
25. Genovese T, Mazzon E, Di Paola R, Muia C, Crisafulli C, Menegazzi M, et al. Hypericum perforatum attenuates the development of cerulein-induced acute pancreatitis in mice. *Shock* 2006; 25: 161-167.