MEDICAL SCIENCE MONITOR

ANIMAL STUDY

e-ISSN 1643-3750
© Med Sci Monit, 2016; 22: 2714-2719
DOI: 10.12659/MSM.896919

Received: 2015.11.30
Accepted: 2016.01.02
Published: 2016.08.01

Effects of Montelukast in an Experimental Model of Acute Pancreatitis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

AB 1 Serkan Angı
AB 1 Hüseyin Eken
AB 1 Erol Kılıç
AB 1 Oktay Karaköse
AB 2 Gürhan Balci
AB 3 Erkan Somuncu

1 Department of General Surgery, Ondokuz Mayıs University, Samsun, Turkey
2 Department of Pathology, Erzincan University, Erzincan, Turkey
3 Department of General Surgery, Erzincan University, Erzincan, Turkey

Corresponding Author: Huseyin Eken, e-mail: huseyineken80@hotmail.com
Source of support: The stud ywas approved by the Ethics Board of Samsun 19 Mayıs University, School of Medicine (Dated 03.07.2008 and issue number 2008/27)

Background: We evaluated the hematological, biochemical, and histopathological effects of Montelukast on pancreatic damage in an experimental acute pancreatitis model created by cerulein in rats before and after the induction of pancreatitis.

Materials/Methods: Forty rats were divided into 4 groups with 10 rats each. The study groups were: the Cerulein (C) group, the Cerulein + early Montelukast (CMe) group, the Cerulein + late Montelukast (CMl) group, and the Control group. The pH, pO2, pCO2, HCO3, leukocyte, hematocrit, pancreatic amylase, and lipase values were measured in the arterial blood samples taken immediately before rats were killed.

Results: There were statistically significant differences between the C group and the Control group in the values of pancreatic amylase, lipase, blood leukocyte, hematocrit, pH, pO2, pCO2, HCO3, and pancreatic water content, and also in each of the values of edema, inflammation, vacuolization, necrosis, and total histopathological score ($P<0.05$). When the CMl group and C group were compared, no statistically significant differences were found in any parameter analyzed. When the CMe group was compared with the C group, pancreatic amylase, lipase, pH, PO2, pCO2, HCO3, pancreatic water content, histopathological edema, inflammation, and total histopathological score values were significantly different between the groups ($P<0.05$). Finally, when the CMe group and the Control group were compared, significant differences were found in all except 2 (leukocyte and pO2) parameters ($P<0.05$).

Conclusions: Leukotriene receptor antagonists used in the late phases of pancreatitis might not result in any benefit; however, when they are given in the early phases or prophylactically, they may decrease pancreatic damage.

MeSH Keywords: Cerulenin • Montenegro • Pancreatitis, Acute Necrotizing

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/896919

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Background

Acute pancreatitis (AP) is an inflammatory disorder characterized by abdominal pain and elevated levels of pancreatic enzymes in the blood, with an annual incidence ranging from 4.9 to 35 per 100 000 [1]. While most of patients have a mild form of this disease, 15–25% develop a severe, necrotizing course of the disease, associated with a high complication rate and a mortality rate of 15–20% [2].

Experimental pancreatitis was first developed by Claude Bernard in 1856 by injecting bile and fatty acids into the pancreatic channel. Thereafter, many experimental studies have been performed to explore the physiopathology of pancreatitis [3–8].

This study aimed to investigate whether Montelukast, a selective cysteinyl leukotriene (CysLT1) receptor antagonist, would have a preventive or healing effect in acute pancreatitis as measured by hematological, biochemical, and histopathological parameters in an experimental acute pancreatitis model developed with cerulein in rats.

Material and Methods

This experimental study was performed in the laboratories of the Ondokuz Mayis University School of Medicine, Medical and Surgical Research Center after obtaining approval from the Ondokuz Mayis University School of Medicine Ethics Board for Animal Experiments. The study was designed according to the Helsinki Declaration. A total of 40 male Sprague-Dawley rats weighing 295–325 g were used in the study.

The 40 rats were divided into 4 groups with 10 rats each. The study groups were; the Cerulein (C) group, the Cerulein + early Montelukast (CMe) group, the Cerulein + late Montelukast (CMl) group, and the Control (CO) group. Cerulein (Sigma Chemical, USA) was injected subcutaneously in each group except the Control group for obtaining pancreatic damage. In the CMe group, Montelukast (Bilim İlaç, Istanbul) was injected subcutaneously in a dose of 10 mg/kg twice, once at 4 h and once at 8 h before the first cerulein dose; and then 20 µg/kg cerulein was subcutaneously injected 4 times at hourly intervals between the doses. In the CMl group, the rats were subcutaneously injected with cerulein in a dose of 20 µg/kg, 4 times, with 1-h intervals between the doses, and then 10 mg/kg Montelukast sodium was injected subcutaneously at 4 and 8 h after the first cerulein dose. In the Control group rats were injected subcutaneously with 0.9% NaCl solution in the same volume as cerulein (0.2 ml) a total of 4 times at 1-h intervals.

Arterial blood samples were taken under anesthesia in all rats 12 h after the first cerulein injection and the rats were killed afterwards. Pancreatic tissue was totally excised for histopathological examination by laparotomy, and arterial blood samples were taken immediately before rats were killed for measurement of the pH, pO2, pCO2, HCO3, leukocyte, hematocrit, pancreatic amylase, and lipase values. Resected pancreatic tissue, divided in 2 groups with approximately 1 cm^3 tissue, was excised from the tail of the pancreas to measure the pancreatic water content. The rest of the pancreatic tissues were histopathologically evaluated for edema, inflammation, vacuolization, and necrosis. Each parameter received a score from 0 to 4, and a total histopathological score was calculated.

Statistical analysis

The data were encoded, then transmitted to a computer and analyzed using the SPSS15.0 (USA) statistics program. Descriptive characteristics of the data are expressed as mean ± standard deviation. The Mann-Whitney U test was used to compare the groups. Statistical significance was considered as $P<0.05$.

Results

Biochemical findings

Amylase and lipase were analyzed in all groups because 2 of the enzymes that are released during pancreatitis from the acinar cells during AP are amylase and lipase, and their concentration in the serum is used to confirm the diagnosis [9].

Pancreatic amylase and lipase values were found to be significantly increased in the C group, CMe group, and CMl group compared with the Control group ($P<0.05$). Pancreatic amylase and lipase values in the CMe group were found to be significantly decreased compared to the C group ($P<0.05$). Pancreatic amylase and lipase values were similar in the CMl group and C group, with no statistically significant differences.

Hematological findings

Leukocyte values were observed to be significantly increased in the C group and CMl group compared to the Control group ($P<0.05$), but there were no significant differences in the CMe group compared to the Control group. Hematocrit values were found to be significantly increased in the C, CMe, and CMl groups compared to the Control group ($P<0.05$). No significant differences were found between the C, CMe, and CMl groups for leukocyte and hematocrit values.

Blood gas analysis

The pH, HCO3, and pCO2 values were significantly different in the C, CMe, and CMl groups compared to the Control group

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Table 1. Schönberg Pancreatitis Scoring Index.

Edema	0 None
	1 Diffuse enlargement in interlobular septae
	2 1 (+) Diffuse enlargement in interlobular septae
	3 2 (+) Diffuse enlargement in interacinar septae
	4 3 (+) Diffuse enlargement in interacinar septae
Inflammation	0 None
	1 Around the ductus
	2 Inside the parenchyma (in <50% of the lobules)
	3 Inside the parenchyma (in 51-75% of the lobules)
	4 Inside the parenchyma (in >75% of the lobules)
Vacuolization	0 None
	1 Periductal (<5%)
	2 Focal (5–20%)
	3 Diffuse (21–50%)
	4 Severe (>50%)
Necrosis	0 None
	1 1-4 necrotic cells*
	2 5–10 necrotic cells
	3 11–16 necrotic cells
	4 >16 necrotic cells

* In microscopic field.

(P<0.05). There were significant differences in the pO2 values in the C and CMl groups compared to the Control group (P<0.05), while they were similar in the CMe and Control groups. When the C and CMe groups were compared, there were statistically significant differences in all pH, HCO3, pCO2, and pO2 values (P<0.05). There were no statistically significant differences in pH, HCO3, pCO2, or pO2 between the C and CMl groups. These results show that Montelukast given before cerulein has positive effects on blood gases; however, Montelukast given after cerulein has no significant effect on blood gases in pancreatitis.

Findings of pancreatic water content

Since an increase in the water content of the pancreas would be expected in acute pancreatitis due to inflammation and edema, pieces of tissues from the tail of the pancreas were taken to evaluate this hypothesis. Pancreatic water content was found to be significantly increased in the C, CMe, and CMl groups compared to the Control group (P<0.05).

Pancreatic water contents were found to be significantly decreased in the C and CMe groups (P<0.05), while they were similar in the C and CMl groups, with no statistical differences between them.

Histopathological findings

Pancreatic tissues were totally resected for histopathological examination in all rats after being killed and were evaluated using the Schönberg Pancreatitis Scoring Index, which gives scores from 0 to 4 for edema, inflammation, vacuolization, and necrosis [10]. The Schönberg Pancreatitis Scoring Index is shown in Table 1.

Each of the histopathological score values of edema, inflammation, vacuolization, necrosis, and total histopathological scores were found to be significantly increased in the C, CMe, and CMl groups compared to the Control group (P<0.05). When the C and CMe groups were compared, the values of edema, inflammation, and total histopathological scores were significantly decreased in the CMe group (P<0.05). There were no significant differences in the vacuolization and necrosis scores between the CMe and C groups. When C and CMl groups are compared, no statistically significant differences were found in the edema, inflammation, vacuolization, and total histopathological scores between the groups. Total histopathological scores (n=10), graphic comparison of histopathological

Table 2. Total histopathological scores according to the groups (n=10 in each group).

	Control	C	CMe	CMl
Edema	3	31	23	31
Inflammation	1	34	28	33
Vacuolization	0	19	16	19
Necrosis	0	10	7	11
Total histopathological score	4	94	74	94

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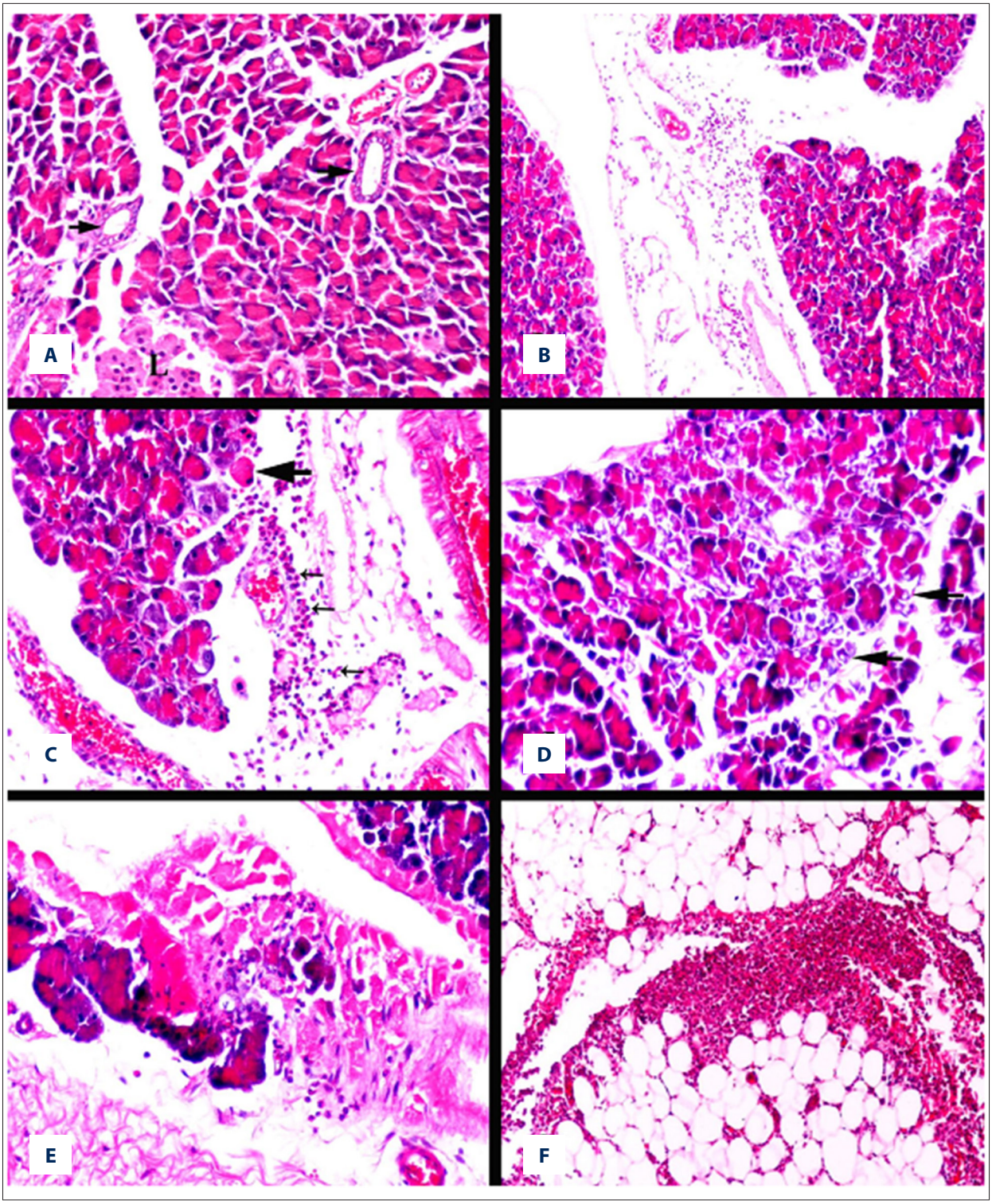

Figure 1. Pancreatic tissue under light microscopy at various magnifications. (**A**) Ductus (arrows) and Langerhans islands (L) between acini in normal pancreatic tissue in the Control group, HE, ×400. (**B**) Inflammation and enlargement in the interlobular septum due to edema in the CMe group, HE, ×200. (**C**) Inflammation in the interlobular septum (narrow arrows) and necrotic changes in the acini (thick arrows) in the CMe group, HE, ×400. (**D**) Vacuolization (arrows) and necrosis in the pancreas in the C group, HE, ×400. (**E**) Pancreatic necrosis and inflammation in the C group, HE, ×400. (**F**) Inflammation in the peripancreatic fat tissue (steatitis) in the CMl group, HE, ×40.

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scores according to the groups, and light microscopy views of tissue samples are shown in Table 2 and Figure 1, respectively.

Discussion

Cerulein, a decapeptid, is isolated from the skin of an amphibian named Hyla (an Australian frog) [11,12]. A cholecystokinin-pancreozymin analog, cerulein was first used by Lampel and Kern to produce experimental acute interstitial pancreatitis in rats in 1977 [13].

Maximal pancreatic damage occurs at 12 h after subcutaneous or intraperitoneal administration of cerulein [14–17]. We administered cerulein subcutaneously 4 times in a dose of 20 micrograms/kg at hourly intervals and we killed the rats at 12 h after the first cerulein dose. The formation of acute pancreatitis was observed in all 10 rats in the group in which only cerulein was administered both macroscopically and histopathologically. Severe edema, inflammation, vacuolization of intermediate severity, and minimal acinar necrotic damage occurred in all rats. These histopathological changes were evaluated according to the Schönberg pancreatitis scoring index and edema, inflammation, vacuolization, and necrosis were scored from 0 to 4 [10]. The degree of damage was assessed according to the total score.

Hotter et al. identified increased edema and polymorphonuclear infiltration in the pancreas of rats that were given leukotriene infusion in hematoxylin eosin staining [18]. Folch et al. demonstrated increased leukotrienes in an experimental model of acute pancreatitis [19].

Leukotrienes cause diffusion of plasma fluid from postcapillary venules to the tissues, leading to edema (chylous ascites). They exert this effect through the activation of receptors specific to themselves [20–24]. After the role of the leukotrienes in the pathogenesis of acute pancreatitis became understood, experimental studies were performed using cysteinyl leukotriene receptor antagonists in the treatment of acute pancreatitis, or used in decreasing severity of or preventing acute pancreatitis [25].

Hirano reported that pranlukast, a cysteinyl receptor antagonist, decreased pancreatic edema in rats and found decreased pancreatic edema and vacuolization in the histopathological evaluation, a decreased microvascular leak, and also a decreased amylase level in rats with pranlukast administration before pancreatitis development with cerulein compared to the control group [26]. Oruc et al., in a different study performed with another leukotriene receptor antagonist, zafirlukast, reported results contradictory to those of Hirano. According to Oruc, a marked increase in the total histopathological score and fat necrosis were observed in the group that was given zafirlukast, but amylase levels were not significantly different [27]. In a study by Ozkan et al., oxidative tissue damage occurring during acute pancreatitis decreased when montelukast was given just before the creation of acute pancreatitis with cerulein [28].

Because the results of the experimental pancreatitis studies performed with leukotriene receptor antagonists conflict, and because there seems to be an obvious need for new studies to clarify the matter, we evaluated the effects of administration of a cysteinyl leukotriene receptor antagonist, montelukast, before and after the induction of pancreatitis on the hematologic, biochemical, and histopathological parameters of pancreatic damage in an experimental acute pancreatitis model induced with cerulein in rats. This was also tested on other gastrointestinal pathologies, such as mesenteric ischemia and pilonidal disease [29–32].

We analyzed the effects of montelukast before and after administering cerulein in different groups. Leukotriene receptor antagonist administration has never been performed after cerulein in pancreatic models, differentiating this study from that of Ozkan et al. The biochemical values and histopathological scores in the group in which montelukast was given before cerulein were similar to the results of Hirano and Ozkan, and significantly different from the values and scores of the group in which only cerulein was given. However, it is not possible to interpret these results as showing a preventive effect of montelukast on the induction of pancreatitis, because there were also significant differences between the montelukast and control group. Thus, the induction of acute pancreatitis was not prevented by montelukast. However, the histopathological score of the pancreatitis was significantly lower than the group with no montelukast administration, showing that the severity of pancreatitis decreased. However, in the group with montelukast administration after cerulein, there were no significant differences in biochemical or histopathological parameters compared to the cerulein group. According to these results, it can be stated that leukotriene receptor antagonist administration after the development of acute pancreatitis has no effect on the prognosis of acute pancreatitis.

Conclusions

The results of our study, when histopathological, biochemical, hematologic, and pancreatic water content measurements are taken into account, show that leukotriene receptor antagonists, used in the late phases of acute pancreatitis, have no effect in treating or decreasing the severity of acute pancreatitis. Although the leukotriene receptor antagonists used before the induction of acute pancreatitis do not prevent the

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development of pancreatitis, in fact, they decrease the severity of pancreatitis that develops later. Use of leukotriene receptor antagonists in the early phases of pancreatitis to decrease the severity of the disease, or prophylactic use before procedures that carry the risk of pancreatitis (e.g., ERCP), is promising.

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Disclosure of conflict of interest

None.

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