Effects of selected antibiotics on pancreatitis induced liver and pulmonary injury

Seçilmiş antibiyotiklerin pankreatitte oluşan karaciğer ve pulmoner hasar üzerine etkileri

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AMAÇ

Pankreatitin erken fazında verilen antibiyotiklerin akut pankreatitin prognostik faktörleri olan karaciğer ve akciğerde oluşan hücresel yıkımı önleyici etkinliğini araştırmak

GİRİŞ

İmipenem ve kinolonlar gibi hem pankreatik doku düzeyleri yüksek hem de yüksek bakterisid etkili antibiyotiklerin akut pankreatittte prognostik faktörler olan karaciğer ve akciğer hasarı üzerindeki etki ve yararlılıklarını tanımlamak için canlı hücre değerlendirmesi ve akut pankreatitte koruyucu rol oynayan plazma nitrik oksit (NO) düzeyleri değerlendirilmiştir.

GEREÇ VE YÖNTEM

Serulein enjeksiyonuyla pankreatit oluşturulan sıçanlara imipenem, levofloksasin ve kontrol grubuna salin verilerek 24. saatte pankreatit oluşturuldu. Serum amilaz, aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), laktik dehidrogenaz (LDH) ve NO düzeyleri ölçülerek pankreatik interstisyel inflamasyon, asiner hücre nekrozu ve vakuolizasyonu, peripankreatik yağ nekrozu, noktasal nekroz ve karaciğerde fokal inflamasyon ile akciğerdeki inflamatuar süreç skorlandı.

BULGULAR

Serum amilaz, AST, ALT ve LDH düzeylerinin antibiyotik gruplarında kontrole daha düşük, imipenem grubunda ise serum NO düzeylerinin levofloksasin ve kontrol grubuna göre daha yüksek olduğu izlendi. Levofloksasin uygulanan grupta pankreasta asiner hücre vakuolizasyonu; akciğerde interstisyel ödem, nötrofil infiltrasyonu ve interstisyel genişleme her iki antibiyotik grubunda ise karaciğerde noktasal nekroz, apoptoz ve fokal inflamasyon skorları daha düşüktü.

SONUÇ

Bu çalışmamızda nekrotizan pankreatitte erken antibiyotik tedavisinin antimikrobiyal etkilerinin yanında pankreas, karaciğer ve akciğerde oluşan hücresel hasar üzerine koruyucu etki göstereceği ortaya çıkmaktadır.

Anahtar Sözcükler: Pankreatit, karaciğer, akciğer, NO, levofloksasin, imipenem

OBJECTIVE

To investigate the protective effect of antibiotherapy in the early phase of acute pancreatitis on cellular injury induced in lungs and liver.

BACKGROUND

Cellular viability and plasma nitric oxide (NO) levels were assessed to determine the efficacy of highly bactericidal imipenem and quinolones on liver and lung injury.

METHODS

Imipenem, levofloxacin or saline were administered to rats with caerulein induced pancreatitis. Twenty-four hours later serum amylase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and NO levels, pancreatic interstitial inflammation, acinar cell necrosis, acinar cell vacuolisation, peripancreatic fat necrosis; spotty necrosis, focal inflammation of liver and inflammatory processes in the lungs were assessed.

RESULTS

Enzyme levels in the antibiotherapy groups were lower than in the control group. Serum NO levels were higher only in the imipenem group. Levofloxacin decreased acinar cell vacuolisation in the pancreas; interstitial edema, neutrophilic infiltration and interstitial enlargement in the lungs. Antibiotherapy decreased spotty necrosis, apoptosis and focal inflammation in the liver.

CONCLUSIONS

Although treatment with imipenem is associated with higher NO levels than levofloxacin, levofloxacin prevents organ injury more effectively than imipenem in acute pancreatitis. Our results indicate that antibiotherapy in the early period of necrotizing pancreatitis prevents cellular damage induced in pancreas, liver and lungs.

Key Words: pancreatitis, liver, lungs, NO, levofloxacin, imipenem

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INTRODUCTION

Acute pancreatitis is a primarily non-bacterial inflammation of the pancreas characterized by abdominal pain and elevation of pancreatic enzymes in plasma and urine. Other local organs and/or organ systems are involved in various degrees in acute pancreatitis and they might lead to different clinical features. Although the disease occurs as a self-limiting mild abdominal pain in most patients it is still associated with a high mortality due to a severe fluid loss, metabolic instabilities, hypotension, sepsis and multiple organ failure in a considerable number of patients. In different series, the mortality rate in acute pancreatitis is reported to be 6-23%.[1,2] The most common cause (95%) of death in the early period is pulmonary complications like pulmonary oedema and congestion. After the first weeks, pankreatitic necrosis, ongoing sepsis and associated multisystem organ failure is the most common (77%) cause of mortality.[2,3,4,5]

Acinar cells synthetize and secrete a variety of inflammatory mediators in the initial phase of acute inflammation. The mediators thus released locally increase vascular permeability, contributing to oedema formation. Rapid release of inflammatory factors results in the accumulation of other chymosin synthesizing cells leading to an extensive inflammatory reaction. Together, these events increase pancreatic oedema and ischemia resulting in pancreatic injury. At the same time, the inflammatory molecules released in the early phase of this process also cause effects like capillary leakage, fever and hypotension. All these events result in pancreatic necrosis, apoptosis and damage in remote organs like lung and liver as well.[6]

NO produced endogenously in the exocrine pancreas, regulates pancreatic secretion and blood flow.[7,8,9] Inhibition of nitric oxide synthetase (NOS) results in reduction of both basal and stimulated pancreatic secretion.[9] This effect can be reversed by administering a NO donor like L-arginin.[9] Though the effect of NO on acute pancreatitis is controversial, it is considered to play a protective role by regulating the microcirculation.[89]

On the other hand various antibiotics have been advocated to decrease infective complications in acute pancreatitis. Since it has been previously demonstrated that carbapenems and quinolones reach high concentrations in pancreatic tissue and distant organs, these two major groups of antibiotics are the first choice antiinfectives in acute pancreatitis.[10]

In this study, we aimed to test the effects of imipenem and levofloxacin administered in the early phase of pancreatitis on the prevention of local (pancreas) and distant (lung and liver) organ injury, as well as on plasma NO levels.

METHODS

Twenty-four male Wistar-Albino rats weighing 250-300 g, purchased from Veterinarian Research Institute, Izmir, were used in this study. Rats were kept in standard animal cages with a free access to rat chow and water before surgery. All of the animal experiments were performed in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health. The animals were anesthetized with an ether inhalation.

Experimental Groups: Rats were separated into four groups. While Sham group (S) (n=6) received intraperitoneal physiologic saline solution, cerulein (100 mcg/kg/dose x 4) was administered intraperitoneally to acute pancreatitis group (AP) (n=6).[11,12] In the acute pancreatitis + imipenem/cilastatin group (AP+IM) (n=6), after the administration of cerulein (100 mcg/kg/dose x 4) intraperitoneally, imipenem/cilastatin was given at a dose of 20 mg/kg bid IV. Finally in the acute pancreatitis + levofloxacin group (AP+LEV) (n=6) after 4 doses of intraperitoneal cerulein at a dose of 100 mcg/kg levofloxacin (20 mg/kg IV bid) was injected after the last cerulein injection.[13] In all groups laparotomy was performed at 24. hours after cerulein injection, blood samples were obtained by cardiac puncture and biopsies of pancreatic, hepatic and lung tissues were taken.

Blood analyses: Serum amylase, AST, ALT and LDH levels were determined using standard assays Blood samples were autoanalysed using Bayer OpeRa automated analyser (Bayer, Germany). Serum NO levels were determined by Nitric Oxide Calorimetric Assay kit (Boehringer, Mannheim, Catalog No : 1756281).

Histological examination . All samples were put into a 10% formaline solution for 24 hours before

processing and embedding in paraffin wax. For routine light microscopic examination (x20, x40, x100), thin liver slices (5 m) were prepared and stained with haemotoxylene-eosine (HE). Pancreatic tissue interstitial inflammation, acinar vacuolisation, acinar necrosis and peripancreatic fat necrosis were scored from 0 to 4 [13,14] according to this the following scoring systems :

Grade 0: Inflammation, vacuolisation or necrosis < 5%

Grade 1: Inflammation, vacuolisation or necrosis 5-15%

Grade 2: Inflammation, vacuolisation or necrosis 15-35%

Grade 3: Inflammation, vacuolisation or necrosis 35-50%

Grade 4: Inflammation, vacuolisation or necrosis > 50%

Liver tissue damage was graded according to spotty necrosis and apoptosis in lobular area under 10x magnification.[15]

0: No focal necrosis, or hepatocyte apoptosis.

1: One focus or less inflammation or hepatocyte necrosis

2: Two to four focal inflammation or hepatocyte necrosis

3: Five to ten focal inflammation or hepatocyte necrosis

4: More than ten focal inflammation or hepatocyte necrosis

Cellular injury in lungs were evaluated for neutrophilic infiltration, interstitial inflammation and enlargement in alveolar septum [14] according to this scoring system :

Grade 0: Absence of interstitial oedema, enlargement and neutrophilic infiltration

Grade 1: Mild interstitial oedema, enlargement and neutrophilic infiltration

Grade 2: Moderate interstitial oedema, enlargement and neutrophilic infiltration

Grade 3: Severe interstitial oedema, enlargement and neutrophilic infiltration

Statistical analyses: All parameters were expressed as mean ± SEM. Statistical analyses were performed with ANOVA test (post-hoc Tukey test) for parametric data, Kruskal Wallis and Mann-Whitney-U tests for histological findings. P<0.05 was considered statistically significant.

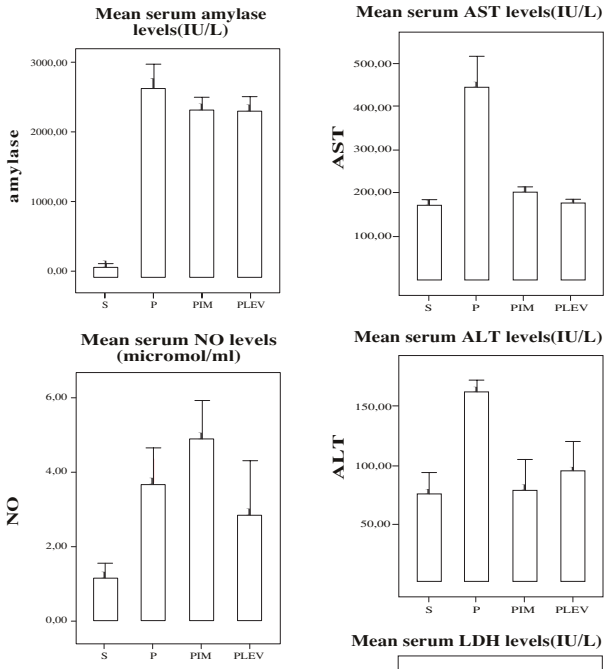

Figure 1. Mean amylase and NO levels in plasma in all groups.

Figure 2. Mean AST, ALT, LDH levels in plasma in all groups.

RESULTS

Table I shows mean (± SEM) values for serum amylase and NO levels in all groups.. Amylase and NO levels were significantly higher in AP, AP+IM and AP+LEV groups than the sham group (p<0.05). This data has been supported by histological findings which also had revealed the presence of acute pancreatitis. However any statistically significant difference was not detected between AP, AP+IM and AP+LEV groups in terms of amylase levels. On the other hand, serum NO level in AP+IM group was higher than AP+LEV group (p<0.05). Any significant difference was not found between AP+LEV / AP and AP+IM / AP groups (Figure 1).

Table II shows mean (± SEM) values for plasma AST, ALT and LDH levels. While AP group demonstrated significant increases in enzyme levels as compared with sham animals (p<0.05), imipen-

Table I. Mean ± SEM serum amylase, NO, AST, ALT and LDH groups in animals (mean ± SEM). All AP animals received antibiotics. Note that the levels are levels similar to those found in the sham group.

RATS	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	AMYLASE (IU/L)	NO (mmol/ml)
S1	143	77	557	137	1,4
S2	193	78	628	162	1,2
S3	121	53	608	162	1,6
S4	178	77	279	204	0,6
S5	190	107	521	100	0,8
S6	217	69	305	105	1,2
Mean ± SEM	**173,66±13,17**	**76,83±6,54**	**483±56,97**	**145±14,67**	**1,13±0,14**
AP1	446	154	8141	2640	3,4
AP2	422	166	5761	2232	3,2
AP3	393	179	13791	2805	5,4
AP4	369	158	13269	2539	3,4
AP5	528	168	8852	2913	3,8
AP6	529	163	9149	3161	2,6
Mean ± SEM	**447,83±25,22**	**164,66±3,24**	**9827,16±1158,85**	**2715±119,73**	**3,63±0,35**
AP+IM1	192	67	4459	2208	5,6
AP+IM2	122	91	4723	2451	4,6
AP+IM3	154	57	4602	2162	4,8
AP+IM4	242	67	4476	2403	3,8
AP+IM5	245	69	4741	2593	4
AP+IM6	252	124	5554	2561	6,4
Mean ± SEM	**201,16±20,24**	**79,16±9,19**	**4759,17±151,7**	**2396,33±66,50**	**4,86±0,36**
AP+LEV1	198	98	1370	2025	4,4
AP+LEV2	124	70	1842	2443	3
AP+LEV3	166	116	1591	2330	4,4
AP+LEV4	242	105	1878	2434	1,2
AP+LEV5	224	123	1975	2593	1,2
AP+LEV6	107	63	1264	2470	2,6
Mean ± SEM	**176,83±20,21**	**95,83±9,09**	**1653,33±108,68**	**2382,50±72,42**	**1,31±0,53**

em-levofloxacin combination was administered. AP rats revealed similar enzyme levels to those seen in the sham group (Figure 2).

Histological findings (acinar cell vacuolisation in pancreas; interstitial oedema, neutrophil infiltration and interstitial enlargement in lungs; spotty necrosis, apoptosis and focal inflammation in the liver) in AP and AP+antibiotic groups are summarized in Table II. While necrosis, apoptosis or inflammation can be seen in the lungs, liver and pancreas, acute pancreatitis results in statistically significant increases in the frequency of these abnormalities when compared with the sham group. Still no significant difference has been observed between AP and AP+antibiotic groups in terms of histological findings.

DISCUSSION

The mechanism responsible for the progression of acute pancreatitis from mere interstitial inflammation to severe acinar cell necrosis is subject to an intense ongoing discussion. It has been suggested that ischaemia and microcirculatory impairment are important factors promoting the development of pancreatic necrosis.[3,6,9] There is an established relationship between NO levels and local pancreatic

Table II. Histologic scores in for pancreas, lungs and liver according to description in text. All AP groups demonstrated more inflammation and necrosis than the sham group, but there were no differences between AP and AP + antibiotic groups.

LIVER	**RAT 1**	**RAT 2**	**RAT 3**	**RAT 4**	**RAT 5**	**RAT 6**
S	1	1	0	1	2	1
AP	3	2	2	3	2	2
AP+IM	2	1	1	2	1	1
AP+LEV	2	1	2	1	0	0
LUNGS	**RAT 1**	**RAT 2**	**RAT 3**	**RAT 4**	**RAT 5**	**RAT 6**
S	1	2	1	1	1	1
AP	2	2	1	3	2	1
AP+IM	2	2	2	2	2	1
AP+LEV	3	2	2	3	3	2
VACUOLIZATION IN PANCREAS	**RAT 1**	**RAT 2**	**RAT 3**	**RAT 4**	**RAT 5**	**RAT 6**
S	1	2	1	1	4	0
AP	1	2	4	3	2	3
AP+IM	2	3	2	1	2	2
AP+LEV	2	1	1	1	0	1
ACINAR NECROSIS IN PANCREAS	**RAT 1**	**RAT 2**	**RAT 3**	**RAT 4**	**RAT 5**	**RAT 6**
S	0	0	0	0	0	0
AP	0	1	3	2	1	1
AP+IM	2	2	0	0	1	1
AP+LEV	2	0	0	0	0	0
INTERSTITIAL INFLAMMATION IN PANCREAS	**RAT 1**	**RAT 2**	**RAT 3**	**RAT 4**	**RAT 5**	**RAT 6**
S	1	0	2	2	0	0
AP	1	2	4	3	3	3
AP+IM	3	3	1	2	3	2
AP+LEV	3	1	2	2	0	2

circulation. in that increased NO levels are associated with a less impaired microcirculation of the pancreas. Thus, increased NO levels may possibly prevent acinar cell necrosis in acute pancreatitis.

Plasma NO levels in acute pancreatitis + imipenem/ cilastatin group were significantly higher than the sham group. In previous studies, imipenem has been found to decrease systemic levels of TNF-α and NO more effectively than other carbapenems and cephalosporins.[16] The changes described in our study are in accordance with these findings.[16] Contrary to this, the interaction between levofloxacine and NO levels in pancreatitis is largely unknown. We have shown that levofloxacine has similar potentializing effects on NO levels like imipenem which protect against acute pancreatitis.

The importance and the validity of this previously summarized feedback mechanism have been demonstrated in many *in vitro* and *in vivo* studies.[17,18,19,20] The inflammatory process can explain hypoproteinemia and increased enzymatic activity of transaminases occurring in the initial phase. Similarly, our study revealed significantly ($p<0.05$) increased AST and ALT levels when compared to those of the sham group. Also these enzyme levels were significantly different between the antibiother-

apy groups and the acute pancreatitis group. However, the inflammatory process can not solely explain LDH increases observed in all subjects with pancreatitis. In antibiotherapy groups these enzyme levels drop into similar levels to those seen in the sham group. We can conclude that both antibiotics we used in this study could reverse hepatic cellular injury in terms of these enzymes.

Acute inflammatory hepatic necrosis and degradation occurring in the initial phase of acute pancreatitis have been demonstrated by Isogai.[21] Carvana et al., also demonstrated that hepatic necrosis takes place n the septic phase.[22] Many investigators have defined the morphologic and histopathologic features of hepatic injury occurring during acute pancreatitis.[21,23] The initial increase in LDH could also be due to this necrotic process. However, in the presence of necrosis and severe pancreatitis, hepatocyte apoptosis occurs as an adaptive manifestation. Imrie and Ranson, back in 1970's, claimed that pancreatitis - associated ascitic fluid (PAAF) that forms intraperitoneally in pancreatitis could play a role in hepatocyte apoptosis.[24] The mechanism of these deleterious effects related to the distant organs such as liver or kidney in severe acute pancreatitis has not yet been fully understood. Early studies demonstrated apoptotic cell death in thymus and kidney during severe acute pancreatitis.[23] It cannot be explained why such an injury does not occur in liver which is an organ that filtrates all the blood coming from the abdominal organs.[23] During pancreatitis hepatic failure is less frequently seen than pulmonary and renal failure. In a study reported by Kyosola et al., the rate of the liver failure in a group of 260 acute and subacute pancreatitis patients was 5%.[23] During acute pancreatitis, the increased levels of LDH can be a heralding sign of latent liver injury.[25] Similarly, in our study, serum LDH levels increased in all pancreatitis groups which correlated with the degree of hepatocellular apoptosis.

Even though not considered as major factors NO and free radicals such as superoxides found in PAAF system might demonstratedly cause apoptosis by altering the mitochondrial oxidation in liver.[23]

Currently, the mechanism of hepatic injury during acute pancreatitis is not clear yet. In our study, the frequency of the hepatocellular apoptosis was higher in acute pancreatitis groups when compared to the sham group. However, the administration of antibiotics altered the degree of apoptosis.

In our study histologic scores of distant organ injury did not reveal any difference between AP and AP+antibiotics groups. This similarity can be explained by distant organ injury caused by local leucocytic infiltration[26,27] under the stimulatory effects of cytokines or enzymes (e.g. elastase, ICAM-1) released from pancreas during acute pancreatitis.

Many studies have been carried out investigating the use of antibiotics in acute pancreatitis. The most crucial of these is a study by Büchler et al., which demonstrated that ciprofloxacin, imipenem and ofloxacin concentrate in the pancreatic tissue during acute pancreatitis.[28] In an open label study, Pederzoli and co-workers achieved imipenem prophylaxis in necrotizing pancreatitis and observed that even though there was no difference in the incidence of multi organ failure between the prophylaxis and the sham group pancreatic and non-pancreatic sepsis rates were found to be significantly different.[29] Authors using a unblinded technique have created a thought provoking bias. For instance, as factors worsening the uninfected necrosis, the impact of the frequency of the surgical intervention and the surgical intervention per se have been ignored. Because of the demonstration of direct effects of carbapenems on NO and TNF levels and the fact that carbapenems and quinolones concentrate in the pancreatic tissue, we examined the pathophysiological effects of these antibiotics on pancreatitis.

According to our results, we can conclude that even though NO levels do not vary among antibiotic-groups and antibiotics have no influence on the pathophysiological course of pancreatitis, they can be used as prophylactic agents against secondary infections. It seems that in the early phase of the necrotizing pancreatitis, initiation of selected antibiotics can help to prevent cellular damage in pancreas, liver and lungs. Imipenem causes higher NO levels than levofloxacin during acute pancreatitis but levofloxacin prevents organ injury more than imipenem.

Imipenem is known to have a differential immunmodulatory effect on lymphocytes and macrophages in humans as well as rodents. Primarily, imipenem

has been found to exert a direct inhibitory effect on T-lymphocytes.[30] Furthermore, imipenem has an immunosuppressive activity on interleukin-2 induced peripheral leukocyte function known as lymphokine-activated killer (LAK)-cell efficacy. [31]

As it is known, T- lymphocytes play a substantial role in determining the severity of acute pancreatitis and inadequate stimulation of these lymphocyte subsets is associated with local and distant organ complications.[32,33] As a consequence, the inhibition of IL-2 mediated T-lymphocyte activation by therapeutic or prophylactic application of the macrolide immunosuppressive agent tacrolimus (FK506) reduced severity of experimental pancreatitis and -like in the current study- not only ameliorated pancreatic damage and acinar cell apoptosis but also prevented distant organ damage.[34]

In this respect, our study opens up a new perspective in the treatment of acute pancreatitis and offers two strong rationale for early antibiotherapy for acute pancreatitis in humans in that antibiotherapy inhibits excessive lymphocyte stimulation by immunomodulatory antibiotics in order to reduce degree of severity and the prevention of bacterial superinfection of pancreatic necrosis in the later phases of the disease.

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