The Effects of Hypertonic Saline Solution, Ascorbic Acid and Low-Molecular-Weight Heparin on Acute Necrotizing Pancreatitis in Rats

Ratlarda Hipertonik Salin Solusyonu, Askorbik Asit ve Düşük Molekül Ağırlıklı Heparinin Akut Nekrotizan Pankreatit Üzerine Etkisi

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Abstract

Objective: We investigated the biochemical and histopathological effects of vitamin C, low-molecular-weight heparin (LMWH), and hypertonic solution on acute necrotizing pancreatitis and on lungs as a terminal organ.

Materials and Methods: We included 48 Sprague-Dawley rats in the study, which were divided into six groups, each with eight rats. The rats in group 1 were sacrificed immediately, in order to determine normal reference values for biochemical and histopathological data. Twenty-four hours after giving intraperitoneal L-arginine to the remaining five groups, development of pancreatitis was shown through assessment of amylase and CRP values. Rats in group 2 were sacrificed at the 24th hour and assigned to the control group for biochemical or the histopathological data groups, in which pancreatitis was induced. The rats in the remaining four groups were given intravenous (IV) isotonic NaCl (group 3), IV vitamin C (group 4), subcutaneous LMWH (group 5), IV hypertonic NaCl (group 6) between 24-48 hours. Each group was assessed with respect to amylase, Serum glutamic oxaloacetic transaminase (SGOT), Lactate dehydrogenase (LDH), C-reactive protein (CRP), bicarbonate, base excess (BE), Ca++, ascorbic acid, and leukocyte at hour 72. Additionally, pancreatic and lung tissue was histopathologically evaluated.

Results: In the treatment groups, amylase and leukocyte levels at the 72nd hour were found to be significantly lower than at the 24th hour ($p<0.05$). The most significant decrease in amylase and leukocyte levels was found in group 6, and damage to the pancreas was found to be lowest in groups 4 and 6.

Conclusion: We observed that in rats, hypertonic NaCl solution and vitamin C reduced the amount of necrosis in the pancreas.

Keywords: Acute necrotizing pancreatitis, Ascorbic acid, Hypertonic solution, Low-molecular-weight heparin.

Özet

Amaç: *Deneysel çalışmamızda vitamin C, düşük molekül ağırlıklı heparin (DMAH) ve hipertonik solüsyonun akut nekrotizan pankreatite ve uç organ olarak akciğerlere biyokimyasal ve histopatolojik etkisini araştırdık.*

Gereç ve Yöntem: *Sprag-Dawley türü 48 sıçan, 8'lik 6 gruba ayrıldı. Grup 1, çalışmanın başlangıcında sakrifiye edilerek biyokimyasal ve histopatolojik verilerin normal referans değerlerini tespit için kullanıldı. Diğer 5 gruba L-arginin verilerek pankreatit oluşturuldu. 24 saat sonra 5 gruptan amilaz ve CRP bakılarak pankreatit geliştiği gösterildi. Grup 2, 24. saatte sakrifiye edilerek pankreatit gelişen sıçanlarda biyokimyasal ve histopatolojik veriler için kontrol grubu yapıldı. Diğer 4 grupta 24-48 saatleri arasında İV izotonik NaCl (Grup 3), İV vitamin C (Grup 4), subkütan DMAH (Grup 5) ve İV hipertonik NaCl (Grup 6) verildi. 72. saatte amilaz, SGOT, LDH, CRP, bikarbonat, baz fazlalığı, Ca++, askorbik asit ve lökosit ölçümü yapıldı; pankreas ve akciğerleri histopatolojik değerlendirildi.*

Bulgular: *Tedavi uygulanan gruplarda 72. saateki amilaz ve lökosit düzeyi 24. saate göre anlamlı derecede düşüktü ($p<0.05$). En fazla düşüklük Grup 6'da gözlenmiştir. Pankreas hasarı en az Grup 4 ve Grup 6'da oluşmuştur.*

Sonuç: *İskemi ve buna bağlı gelişen inflamasyon sonucunda ortaya çıkan serbest oksijen radikalleri ve immün mediyatörler pankreas hasarına yol açmaktadır. Yaptığımız çalışmada hipertonik solüsyonların ve vitamin C'nin pankreasta nekroz gelişimini azalttığı gösterilmiştir. Elde ettiğimiz verilere dayanarak, ciddi akut nekrotizan pankreatitin tedavisinde, hipertonik tuz solüsyonları ve vitamin C'nin etkili olabileceği sonucuna vardık.*

Anahtar Kelimeler: *Akut nekrotizan pankreatit, Askorbik asit, Düşük molekül ağırlıklı heparin, Hipertonik solüsyon*

Introduction

Acute pancreatitis is a reversible, inflammatory disease. This disease varies in severity from simple edema to morphologic necrosis. In most patients with acute pancreatitis the disease is trivial, characterized by interstitial edema of the pancreas. However, more severe forms of the disease exist in which patients experience gross pancreatic or peripancreatic necrosis [1-3].

The clinical picture of pancreatitis is that of inflammation and occurs as a result of self absorption of tissue due to the activation of pancreatic enzymes [4, 5]. Pancreatic enzymes, proenzymes transformed into active enzymes, destroy acinar cells and stimulate the compliment system by releasing cytokines. Thus, inflammatory cells, especially neutrophils, are activated and release additional agents including cytokines, free oxygen radicals, and nitric oxide. Many injuries caused by free oxygen radicals involving the vein structure of the pancreas and acinar cells are responsible for initiating acute interstitial and necrotizing pancreatitis [6-9].

Hypertonic and hyperoncotic liquids have been shown to improve heart contractility and pulmonary function in many experimental studies. Hypertonic solutions prevent neutrophil migration by reducing endothelial permeability of veins and thus lowering the potential for inflammation in target organs [2, 10, 11].

Low-molecular-weight heparins (LMWH) have anti-inflammatory effects, preventing microthromboses and increasing microcirculation. These effects stem from influences that enhance capillary vasodilatation and permeability, and inhibit platelet aggregation; anticoagulant properties also contribute to the effect. Furthermore, LMWHs cause free oxygen radicals to move away from the environment. In numerous experimental studies, heparin has been shown to have a direct inhibitory effect on pancreatic tissue and pancreatic proteases in the plasma, as well as increase pancreatic microcirculation. In one clinical study, the development of pancreatitis associated with endoscopic retrograde cholangiopancreatography (ERCP) was shown to be quite low in patients who took heparin or LMWH prior to the procedure [2,12].

Ascorbic acid from vitamin C is the most powerful antioxidant in human blood. Vitamin C taken via diet is absorbed as ascorbic acid in the small intestine. In some studies, the ratio between vitamin C and ascorbic acid has been found to be decreased during acute pancreatitis [13]. The paucity of ascorbic acid raises the sensitivity of the pancreas to free oxygen radicals [13].

Our aim was to investigate the impact of hypertonic solution, ascorbic acid, and low-molecular-weight heparin on acute necrotizing pancreatitis in rats.

Table 1. Groups

Group	Description	Time of sacrifice
1	Control	Hour 0
2	Pancreatitis	Hour 24
3	Pancreatitis + IV NaCl Solution	Hour 72
4	Pancreatitis + IV Vitamin C	Hour 72
5	Pancreatitis + LMWH* (subcutaneous)	Hour 72
6	Pancreatitis + IV Hypertonic NaCl Solution	Hour 72

Table 2. Pancreatic and pulmonary injury scoring systems

Pancreatic injury score		
Parameter	**Finding**	**Score**
Edema	Does not exist	0
	Focal	1
	Extensive	2
Inflammatory cell infiltration	Does not exist	0
	<%50	1
	>%50	2
Parenchymal necrosis	Does not exist	0
	Focal	1
	Extensive	2
Periparenchymal inflammation and necrosis	Does not exist	0
	Preductal	1
	Focal	2
	Extensive	3

Pulmonary injury score		
Parameter	**Finding**	**Score**
Athelectasis or emphysema	Does not exist	0
	Focal	1
	Extensive	2
Alveolar bleeding	Does not exist	0
	Focal	1
	Extensive	2
Congestion	Does not exist	0
	Focal	1
	Extensive	2
Intraalveolar bleeding	Does not exist	0
	Focal	1
	Extensive	2
Interstitial polymorphic leucocyte	Does not exist	0
	Focal	1
	Extensive	2
Hyalen membrane	Does not exist	0
	Focal	1
	Extensive	2

Materials and Methods

In our study, 48 male Sprague-Dawley rats weighing between 200-300 g were utilized. The rats were provided by the Experimental Animal Production and Research Laboratory, Medical Faculty of Istanbul University. The rats were randomly divided into six groups, each comprised of eight rats (Table 1). Rats were given standard laboratory food and tap water during the course of the study.

At the beginning of the study, the chests of eight rats were opened under ether anesthesia in order to collect 4 ml of blood from their hearts (Group 1). Control reference values were established for amylase, Serum glutamic oxaloacetic transaminase SGOT, Lactate dehydrogenase (LDH), ascorbic acid, C-reactive protein (CRP), Ca++, bicarbonate, base excess (BE) and leukocyte count by biochemical analysis. Intraperitoneal L-arginine (500 mg/100 g) was injected into the remaining five groups to induce pancreatitis. The rats in group 2 were sacrificed after 24 hours and 4 mls of blood were obtained. The initial control values for the groups in which pancreatitis was induced, were obtained by examining amylase, SGOT, LDH, ascorbic acid, CRP, Ca++, bicarbonate, BE, and leukocyte count via biochemical analysis in group 2. Amylase and CRP were measured at hour 24 from blood sam-

Table 3. Levels of the biochemical parameters which were obtained from group 1

Parameter	Levels (mean±SD)
Amylase	884±184 IU/l
CRP	0.13±0.07 mg/dl
SGOT	203±39 IU/l
LDH	990±267 IU/l
Ca^{++}	0.98±0.2 mmol/l
$HCO3^-$	19.9±2.2 mmol/l
Base excess	- 0.97 mmol/l
Ascorbic acid	1.25±0.16 mg/dl
Leukocyte	9933±3351/mm^3

ples from the rats in the remaining four groups with pancreatitis and four different treatments were applied between the 24th-48th hours. Group 3 received an intravenous (IV) isotonic sodium chloride (0.9% NaCl, 0.2 ml/100 g) infusion, group 4 IV vitamin C (Redoxon ampule, 0.2 ml/100 g), group 5 a subcutaneous LMWH (Enoxaparin, 0.1 mg/100 g) injection, and group 6 an IV hypertonic sodium chloride (7.5% NaCl, 0.2 ml/100 g) infusion. The penile vein was used to administer treatment and for collecting blood. At hour 72, the chests of the rats were opened under ether anesthesia and 4 mls of blood were drawn. Amylase, SGOT, LDH, ascorbic acid, C-reactive protein, Ca++, bicarbonate, BE, and leukocyte count were determined by biochemical analysis.

All rats were decapitated after blood was drawn. For histopathological examination, lung and pancreas tissues were removed, fixed in 10% formalin solution and sent to the department of pathology. Single-blind examination of tissues was performed by a single pathologist who assessed tissue for pancreas and lung impairment. Hematoxylin-eosin staining preparations were studied under light microscopy and scored according to previously established pancreas and lung impairment criteria (Table 2).

All biochemical assessments were carried out in the Emergency Biochemical Laboratory and Central Biochemical Laboratory of Medical Faculty of Istanbul University. Amylase, SGOT, LDH, CRP and ascorbic acid levels were determined from sera while bicarbonate, BE and Ca++ levels were found by blood gas analysis in heparinized blood.

Statistical analyses were performed using the Graphpad Prisma v.3 packet program. Wilcoxon, Kruskal-Wallis, and chi-square tests were applied for repeated measurements (24 hours – 72 hours), intergroup comparison, and qualitative data comparison, respectively. The results are reported as mean ± standard deviation. A p value of < 0.05 was considered statistically significant.

Results

Reference values were obtained from biochemical parameters of rats in group 1 (Table 3). To show L-arginine-induced pancreatitis, amylase and C-reactive protein levels were assessed after 24 hours from rats in groups 2, 3, 4, 5, and 6 (Table 4). Amylase values of groups 2, 3, 4, 5, and 6 were found to be significantly higher than those of group 1. There was no statistically significant difference in terms of CRP values.

Amylase and CRP values from groups 3, 4, 5, and 6 were reassessed at hour 72 and were compared with amylase and CRP values from hour 24 (Table 4). Amylase values from groups 3, 4, 5, and 6 at the 72nd hour were significantly lower than those at the 24th hour. The amylase values of groups 4 and 6 at the 72nd hour were significantly lower than those of groups 3 and 5. There was no statistically significant difference with respect to CRP.

Table 4. Amylase and CRP levels in groups 2, 3, 4, 5 and 6 at the 24th hour; in groups 3, 4, 5 and 6 at the 72nd hour

	Amylase (IU/l)			*CRP (IU/l)*		
Group	**Hour 24**	**Hour 72**	***P***	**Hour 24**	**Hour 72**	***P***
2	1495±414*			0.10±0.05		>0.05
3	1667±658*	651±81	<0.05	0.10±0.03	0.13±0.03	>0.05
4	1342±347*	557±90⁺	<0.05	0.09±0.05	0.09±0.05	>0.05
5	1606±439*	769±229	<0.05	0.14±0.06	0.08±0.02	>0.05
6	1647±356*	484±127⁺	<0.05	0.14±0.05	0.12±0.05	>0.05

* Statistically significantly higher than Group1 (p<0.01)
⁺ Statistically significantly lower than Group 3 and 5 (p<0.001)

Leukocyte count was compared among groups with induced pancreatitis (groups 2-6) at hour 72 (Table 5). Using leukocyte values from group 2 as a baseline, leukocyte counts for all treatment groups were established to be statistically significantly reduced. This was especially apparent in groups 4 and 6.

At hour 72, Bicarbonate (HCO3-), BE, Ca++, SGOT, LDH, and ascorbic acid values were compared between groups receiving therapy and group 2 (Table 6). Bicarbonate and BE values of groups 3, 5, and 6 were significantly higher than those of the control group (group 2). However, there was no significant difference between group 4 and the control group. Compared to Ca++ values of the control group (group 2), Ca++ values in all treatment groups were considerably higher at the 72nd hour. SGOT and LDH values at hour 72 in treatment groups were comparable with those of the control group (Table 6). SGOT and LDH levels of all treatment groups were significantly lower than those of group 2, and although there was no significant difference between treatment groups, the lowest values were found in group 6. Ascorbic acid levels at the 72nd hour, in treatment groups, were analogous with those of the control group (Table 6) with ascorbic acid levels found to be highest in group 4.

Pancreas and lung damage scores of all groups were determined and mean damage scores were compared (Table 7). The pancreatic damage score of group 6 was found to be significantly lower than those of other treatment groups (groups 3, 4, 5, and 6). No difference was detected between groups with respect to lung damage score.

Table 5. Comparison of leukocyte levels of the treatment groups and pancreatitis group (Group 2) at the 72nd hour

Group	Leukocyte count (/mm^3)
2	24500±5200
3	19225±5330*
4	9633±1076*
5	17466±2265*
6	10916±1566*

* Statistically significantly lower than group 2 (p<0.001)

Table 6. Comparison of the HCO_3^- and BE, Ca^{++}, SGOT, LDH and ascorbic acid levels in treatment groups and group 2 at the 72nd hour

Group	HCO_3^- (mmol/l)	BE (mmol/l)	Ca^{++} (mmol/l)	SGOT (IU/l)	LDH (IU/l)	Ascorbic acid (mg/dl)
2	14±3	-3.2±0.5	0.81±0.11	1534±376	2240±742	0.71±0.18
3	27±3*	6.2±3.5*	1.02±0.14+	435±197&	1100±453&	0.68±0.16
4	19±2	1.6±9.4	0.99±0.13+	549±232&	1170±508&	1.16±0.18+
5	28±3*	4.8±3.6*	1.04±0.12+	494±377&	1100±621&	0.73±0.21
6	28±3*	8.9±5.1*	1.21±0.05+	340±208&	850±272&	0.96±0.14

* Statistically significantly higher than group 2 ($p<0.05$)
+ Statistically significantly higher than group 2 ($p<0.01$)
& Statistically significantly lower than group 2 ($p<0.001$)

Discussion

Alcoholic pancreatitis was described by Fitz and Freidrich in 1888 and 1889, respectively, and Opie described biliary pancreatitis in 1901. Despite an increase in knowledge and experience, the optimal treatment is still controversial.

High-dose-L-arginine has been shown to produce damage to acinar lung cells by inhibiting synthesis of nucleic acid and protein [14]. The advantage of this method is that it is noninvasive and easily repeatable, and thus helps researchers carry out studies on the pathophysiology and potential treatments of the disease [14, 15]. We used an intraperitoneal L-arginine injection method to produce a model of experimental acute necrotizing pancreatitis.

During acute pancreatitis, an increase in amylase appears 2-12 hours after the first acute pancreatitis attack and appearance of clinical symptoms and reaches a peak between 12-72 hours. The value generally returns to normal in cases without complications [2, 5, 16, 17]. Serum amylase levels showed significant increases in groups 2, 3, 4, 5 and 6 at the 24th hour of induced acute pancreatitis in this study. Significant regression of amylase levels was observed in treatment groups (group 3, 4, 5, and 6) at hour 72. The most noteworthy regression was in the group receiving hypertonic NaCl solution. This result differed from a previous study in which no significant difference was found in serum amylase levels at the 72nd hour between study groups receiving hypertonic and isotonic solution [11].

Many organ systems are affected during a systemic inflammatory response, during which serum levels of acute phase reactants (cytokines, interleukin-1, tumor necrosis factor-alpha, amyloid-A, C-reactive protein, complement C3, alpha-1 acid glycoprotein, fibrinogen, haptoglobulin and alpha-1 antitrypsin) are elevated [18]. CRP is the most important acute phase reactant and is used to evaluate the severity of the inflammation and disease in acute necrotizing pancreatitis [19]. While an increase in CRP is often found to be significant in many clinical studies looking at acute pancreatitis, a number of studies have shown no significant increase [18-20]. We did not find any significant changes between CRP levels in groups with induced pancreatitis (groups 2, 3, 4, 5, and 6) at hour 24, nor in treatment groups (groups 3, 4, 5, and 6) at hour 72.

Leukocyte levels are known to rise during acute necrotizing pancreatitis [2]. Significant enhancements are seen in blood leukocyte counts whenever areas of necrosis are present. A predominance of neutrophils is seen with the elevated leukocyte level [2, 7, 21]. Within our study, blood leukocyte levels rose significantly in all groups with induced pancreatitis. The highest leukocyte level was observed in the control group (group 3) and the lowest in groups given vitamin C and hypertonic solution.

Significant differences were present between the control group receiving isotonic solution (group 3) and treatment groups with respect to bicarbonate levels and BE.

Hypercalcemia is observed secondary to hyperparathyroidism during acute pancreatitis [22]. Despite its unknown mechanisms, calcium deposits are seen in half of patients with pancreatitis. Calcium plays an important role in the activation of pancreatic proenzymes as well as in synthesis and secretion from acinar cells. As the disease advances, hypercalcemia develops as a result of active calcium metabolism, consequently calcium is deposited in impaired tissue [22]. No significant difference was seen between treatment groups and group 1 in terms of Ca++ levels. The calcium decrease in acute pancreatitis groups and the non-occurrence of an expected increase in the treatment groups can be explained by the shortness of our follow-up period after (72 hours) the development of acute pancreatitis.

In acute pancreatitis, LDH levels increase due to damage to pancreas acinar cells and are a prognostic factor in acute pancreatitis [2, 23]. LDH levels of all groups with induced pancreatitis were significantly higher than controls in our study ($p<0.05$). There was no significant difference between each treatment group (groups 3-6) and the pancreatitis control group (group 2).

Serum transaminase levels are of great importance in determining the etiology of pancreatitis. However, serum transaminase levels in patients with acute pancreatitis can rise due to edema and pressure on the bile duct [2, 24]. SGOT levels of all groups with acute pancreatitis were significantly elevated in our study. SGOT levels in treatment groups were significantly diminished. The highest SGOT value was seen in the group given vitamin C, while the lowest was in the group who received hypertonic solution.

It has been determined that ascorbic acid is the active form of vitamin C and that it is the strongest antioxidant in human blood. Ascorbic acid is effective at inhibiting the development of hemorrhagic and necrotizing pancreatitis by causing inactivation of free radicals in the pancreas. Ascorbic acid levels decrease during acute pancreatitis though the reason is unclear. It may be the result of usage against free oxygen radicals [13, 25]. Within our study, ascorbic acid levels decreased in all treatment groups apart from the group given vitamin C. It seems that serum ascorbic acid level decreases with the onset of acute necrotizing pancreatitis. When administered, vitamin C is transformed into its active form and raises serum levels. Many experimental and clinical studies

Table 7. Mean pancreatic and pulmonary injury score

Group	Mean pancreatic injury score	Mean pulmonary injury score
1	1.3	3.3
2	3.0	8.6
3	7.1	8.6
4	6.2	8.5
5	8.2	8.7
6	5.0*	9.7

* Statistically significantly lower than group 3, 4 ve 5 ($p<0.05$)

suggest that ascorbic acid and vitamin C are present at a certain ratio and that the amount of ascorbic acid functioning as an antioxidant diminishes rapidly [13, 25].

Histopathologic changes observed during an acute pancreatitis attack can be studied in two stages - early and late. In the early stage, acinar cells are introduced to factors that initiate zymogen as well as facilitate the release of inflammatory mediators and molecules affecting vascular permeability. These, in turn, lead to ischemic events in the gland, resulting in edema. Increased inflammatory mediator release starts a cycle which causes inflammatory cells producing cytokines to release the cytokines, further exacerbating the inflammatory response. The sequence of events induces ischemic changes in the gland while enhancing edema [26]. We scored pancreas tissue samples from subjects sacrificed at hour 72 in terms of edema, inflammatory cell infiltration, acinar necrosis and peripancreatic inflammation, and necrosis. The lowest pancreas damage scores were seen in groups 4 and 6.

The lungs of the rats were evaluated histopathologically in order to investigate terminal organ damage. We assessed lung tissue with respect to atelectasis, emphysema, alveolar bleeding, congestion, the presence of cells inside alveoli, interstitial PNL, and hyaline membrane formation. No difference was found between treatment groups in terms of lung damage. The highest score was seen in group 6.

In our study we demonstrated that hypertonic solution and vitamin C reduce the development of necrosis in pancreatitis. Although it is expected that LMWH treatment would cure microcirculation issues and reduce the development of necrosis during pancreatitis, not difference from that of the control group was found. Based on our data, we concluded that for the treatment of acute necrotizing pancreatitis in rats, hypertonic salt solution and vitamin C are effective treatments in the early stage of the disease.

Conflict interest statement The authors declare that they have no conflict of interest to the publication of this article.

References

1. Leach SD, Gorelick FS, Modlin JM. Acute pancreatitis at its centenary. The contribution of Reginald Fitz. Ann Surg 1990; 19: 779-86.
2. Yeo CJ, Cameron JL. The pancreas. In Sabiston DC (ed): Sabiston Textbook of Surgery. Philadelphia, W.B. Saunders, 16th edition, 2001; 1116-25.
3. Ho HS, Frey CF. Gastrointestinal and pancreatic complications associated with severe pancreatitis. Arc Surg 1995; 130: 817-23.
4. Agarwal N, Pitschumoni CS. Assesment of severity in acute pankreatitis. Am J of Gastroenterology 1991; 86: 1385-91.
5. Carfield AP, Cooper MJ, Williamson RCN. Acute pancreatitis. A lethal disaese of increasing incidence. Gut 1985; 26: 724-6.
6. Acioli JM, Isobe M, Kawasaki S. Early complement system activation and neutrophly priming in acute pancreatitis: Parcipiation of trypsin. Surgery 1997; 122: 909-17.
7. Mora A, Perez Mateo M, Viedma JA, et al. Activation of celluler immune response in acute pancreatitis. Gut 1997; 40: 794-7.
8. Schoenberg MH, Buchler M, Gaspar M, Stinner A, Younes M. Oxygen free radicals in acute pancreatitis. Gut 1990; 31: 1138-48.
9. Schulz HU, Niederau C, Klonowsky-Stumpe H, Halangk W, Luthen R, Lippert H. Oxidative stress in acute pancreatitis. Hepatogastroenterology 1999; 46: 2730-50.
10. Chen HM, Sunamura M, Shibuya K, et al. Early microcirculatory derangement in mild and severe pancreatitis models in mice. Surg Today 2001; 31: 634-42.
11. Shields CJ, Winter DC, Sookhai S, Ryan L, Kirwan WO, Redmond HP. Hypertonic saline attenuates end-organ damage in an experimental model of acute pancreatitis. Br J Surg 2000; 87: 1336-40.
12. Framke B, Martus B, Fischer B, Nusko G. Complications of endoscopic sphincterotomy:can heparin prevent acute pancreatitis after ERCP? Gastro Endosc 2002; 55: 476-83.
13. Scott P, Bruce D, Schofield D, Shiel N, Braganza JM, McCloy RF. Vitamin C status in patients with acute pancreatitis. Br J Surg 1993; 80: 750-4.
14. Tani S, Itoh H, Okabayashi Y. New model of acute necrotising pancreatitis induced by excessive doses of argine in rats. Dig Dis Sci 1990; 35: 367-74.
15. Czako L, Takacs T, Varga IS. Involvement of oxygen derived free radicals in l-Arginine induced acute pancreatitis. Dig Dis Sci 1998; 43: 1770-7.
16. Howard RA. Pancreas. In: Schwartz SI (ed). Principles Surgery. 7th ed. McGraw Hill Company, Volume 2, 1999; 1467-99.
17. Schmidt J, Fernández-del Castillo C, Rattner DW, Lewandrowski KB, Messmer K, Warshaw AL. Hypertonic ultrahigh molecular weight dextran solutions reduce trypsinojen activation, prevent aciner necrosis, and lower mortality in rodent pancreatitis. Am J Surg 1993; 165: 40-4.
18. Imamura T, Tanaka S, Yoshida H, Kitamura K. Significance of measurement of high-sensivity C-reactive protein in acute pancreatitis. J Gastroenterol 2002; 37: 935-8.
19. Roumen MH, Meurs PA, Kuypers HHC. Serum IL-6 and CRP responses in patients after laparascopic or conventional cholesystectomy. Eur J Surg 1992; 158: 541-4.
20. Cho JM, La Porta AJ, Clark JR, et al. Response of serum cytokines in patients undergoing surgery. Surg Endosc 1994; 8: 1380-4.
21. Reber HA. Alcholic pancreatitis: In Hovard JM, et al. (ed). Surgical dissease of the pancreas. Washington, DC. Febingers Inc. 1987; 284-96.
22. Reber AH. Pancreas. In: Schwartz SI (ed). Principles Surgery. 7th ed. McGraw Hill Company, Volume 2, 1999; 1467-99.
23. Fomella LS, Galloway W. Inflamatory mediators in acute pancreatitis. Br. J. Surg 1995; 82: 6-13.
24. Ohzato H, Yoshizaki K, Nishimoto N. IL-6 as a new indicator of inflammatory status after surgery. Surgery 1992; 11: 201-9.
25. Sodeman W. Pathologic and normal physiology of the liver. In: Sodeman W, Sodeman T (eds). Pathologic Physiology (7th edition). WB Saunders Company 1985; 954-91.
26. Bonham MJD, Abu-Zidan FM, Simovic MO, Wilkinson KB. Early ascorbic acid depletion is related to the severity of acute pancreatitis. Br J Surg 1999; 86: 1296-301.