SARI RÄTY

The Role of Gallbladder, Cholecystokinin and Microbes in Gallstone and Post-ERCP Pancreatitis

University of Tampere
Tampere 2000

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ACADEMIC DISSERTATION
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ACADEMIC DISSERTATION

To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the small auditorium of Building B,
Medical School of the University of Tampere,
Medisiinarinkatu 3, Tampere, on December 8th, 2000, at 12 o'clock.

University of Tampere
Tampere 2000

To my family

CONTENTS

LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following publications:

I Räty S, Jaakkola M, Karjalainen J, Kuivanen H, Sand J, Nordback I (1997). The presence of the gallbladder is associated with the severity of acute biliary pancreatitis. Int J Pancreatol 21: 145-148.

II Räty S, Sand J, Laine S, Harmoinen A, Nordback I (1999). Cholecystokinin in the early course of acute post-ERCP pancreatitis. J Am Coll Surg 189: 560-565.

III Räty S, Sand J, Kemppainen E, Laine S, Nordback I (2000). Cholecystokinin in acute alcoholic and biliary pancreatitis. Int J Pancreatol 28: 53-59.

IV Räty S, Sand J, Nordback I (1998). Difference in microbes contaminating pancreatic necrosis in biliary and alcoholic pancreatitis. Int J Pancreatol 24: 187-191.

V Räty S, Sand J, Matikainen M, Pulkkinen M, Nordback I. Post-ERCP pancreatitis: reduction by routine antibiotics. Submitted.

In addition, some previously unpublished investigations have been described.

ABBREVIATIONS

AFOS	alkaline phosphatase
ALAT	alanine aminotransferase
AMYL	amylase activity
BD	bile duct
BIL	total bilirubin
CBD	common bile duct
CCK	cholecystokinin
CGRP	calcitonin gene-related peptide
CRP	C-reactive protein
CT	computed tomography
ERCP	endoscopic retrograde cholangiopancreatography
EST	endoscopic sphincterotomy
LEUK	blood leukocyte count
NS	no significance
PD	pancreatic duct
PHI	peptide histidine-isoleucine
PYY	peptide YY
SO	sphincter of Oddi
VIP	vasoactive intestinal polypeptide

INTRODUCTION

Acute pancreatitis is a common disease with an increasing incidence (Corfield et al. 1985, Jaakkola and Nordback 1993, McKay et al. 1999). In Finland the incidence has been reported to be as high as 70 per 100 000 population per year, causing 3000 hospital treatment periods every year (Jaakkola and Nordback 1993). Most episodes of acute pancreatitis are mild and self-limiting, but a fifth of all pancreatitis patients develops a severe disease with long hospital and intensive care unit stays, with high morbidity and mortality (De Beaux et al. 1995). In Finland the mean hospital stay among necrotizing pancreatitis patients is 40 days and intensive care unit stays range from 13 to 24 days (Sainio et al. 1995). Although, the mortality of necrotizing pancreatitis has fallen during the past few decades, it still is up to 6-10 % (Bourke 1977, Lankisch et al. 1996), the most severe episodes having a much higher mortality.

Gallstones and alcohol are the most common etiological factors of acute pancreatitis (Jaakkola and Nordback 1993, Lankisch et al. 1996). In Finland the proportion of acute pancreatitis attributed gallstones is 15 per cent and alcohol 70 per cent (Jaakkola and Nordback 1993), whereas in the United Kingdom, Germany, Hong Kong and in parts of America gallstone disease is the most common cause of acute pancreatitis accounting for 40 to 50 per cent of cases (Corfield et al. 1985, Lankisch et al. 1996, Fan et al. 1988, Jacobs et al. 1977). There is also evidence that a part of so-called idiopathic pancreatitis is caused by gallstones or biliary sludge (Ros et al. 1991, Lee et al. 1992). The pathogenesis of gallstone pancreatitis has been studied for 100 years (Opie 1901), nevertheless, the mechanisms which mediate pancreatitis episodes are still unclear and therefore, the options for specific treatment are limited.

Rare etiologies of acute pancreatitis are connected with endoscopic retrograde cholangiopancreatography (ERCP), trauma, drugs, other diseases and heredity, ERCP being the most common of those (Marshall 1993). Most post-ERCP pancreatitis episodes are mild, but 0.5 % of post-ERCP pancreatitis develop into a severe, necrotizing disease (Nordback and Airo 1988). Whilst, some correlations of different patient or procedure-related risk factors of post-ERCP pancreatitis have been found, the initial mechanism is still not fully understood.

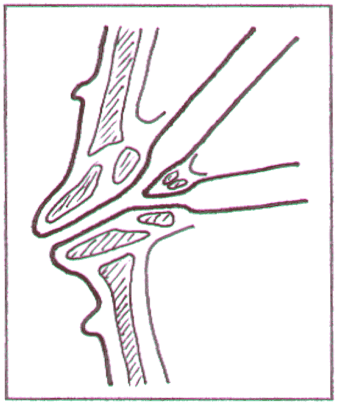
The purpose of this study was to investigate the role of the gallbladder, cholecystokinin (CCK) and microbes in the pathogenesis of human acute gallstone and post-ERCP pancreatitis.

REVIEW OF THE LITERATURE

1 Anatomy of biliopancreatic tree

The biliary tree consists of hepatic ducts, cystic duct, gallbladder, common bile duct and the sphincter of Oddi (SO) in many species including man (Grace et al. 1990). In man three main categories exist at the termination of the bile and pancreatic ducts: (1) the two ducts join to form a common channel, (2) the common bile duct (CBD) and pancreatic duct (PD) are distinct but share a common entrance into the duodenum and (3) the bile duct (BD) and PD open separately into the duodenum (Mann et al. 1920). The first alternative, the common channel anatomy, predominates in man. Among acute gallstone pancreatitis patients the common channel is found in 70 per cent of cases (Uomo et al. 1993).

The majority of the population have one main PD. The incidence of pancreas divisum, allowing a part of the pancreatic juice flow through the minor papilla into the duodenum, is 10 % in the general population (Bernard et al. 1990). However, pancreas divisum is found in 20-50 % of the patients with idiopathic pancreatitis (Neoptolemos et al. 1988, Bernard et al. 1990), whereas its role in acute gallstone pancreatitis remains controversial (Nowak et al. 1990, Uomo et al. 1993). Instead, other congenital disorders, such as high junction of the CBD and PD have an association with the increased incidence of acute pancreatitis (Misra and Dwivedi 1990, Tian et al. 1995, Sugiyama and Atomi 1998). In such cases, obstruction of the PD by a stone or bile reflux through the dilated common channel can precipitate pancreatitis (Greene et al. 1985).



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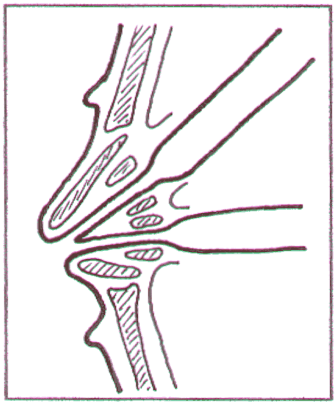

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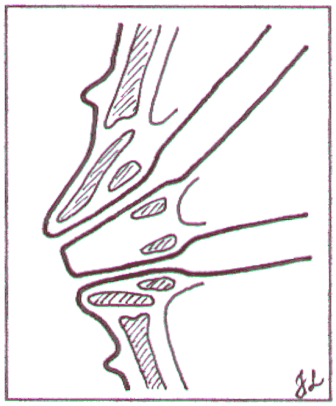


3.

2 Motility of gallbladder and biliary tree

2.1 Physiological conditions

The motility of the gallbladder can be divided into two periods: digestive and interdigestive period (Ryan 1991). In the digestive period, the gallbladder is contracted in response to a meal entering the duodenum (Thomson et al. 1975), whereas the SO is relaxed, both resulting in the increased bile flow into the duodenum (Grider and Makhlouf 1987). Simultaneously, pancreatic enzyme secretion into the duodenum is enhanced leading to the digestion of the meal (Liddle et al. 1985). The contraction of the gallbladder and the relaxation of the SO involves the interaction of neural, hormonal and local factors (Grace et al. 1990, Sand et al. 1993). In the interdigestive period, the function of the SO allows intermittent delivery of the bile into the duodenum (Itoh et al. 1982, Müller et al. 1984). This bile is not only the hepatic bile, it has been shown that the gallbladder is also partially emptying in the interdigestive period (Svenberg et al. 1982).

2.2 Neural control of gallbladder

Neural control of gallbladder contraction is mainly mediated by cholinergic vagal neurones. Sham feeding stimulates gallbladder emptying in up to 50 % of people and this response is eliminated by cholinergic blockade with atropine, suggesting that the vagus can stimulate gallbladder contraction (Fisher et al. 1986). Also, after truncal vagotomy, the postcontraction volume of the gallbladder is greater (Inberg and Vuorio 1969). While cholinergic vagal stimulation alone can effect gallbladder function, some peptide hormones have also been shown to effect the gallbladder function via same parasympathetic neurones (Strah et al. 1986).

2.3 Effects of peptide hormones and neuropeptides on gallbladder function

Peptide hormones are a group of peptides that regulate the motility, secretion, circulation and absorption of the gallbladder and bowel (Grace et al. 1990). The release of these peptide hormones is regulated humorally by circulation (endocrine cells) and/or by peripheral and central neurones (Ryan 1991). CCK was the first peptide hormone isolated in 1966 (Jorpes and Mutt 1966). CCK is released from the duodenum by luminal acid and nutrients resulting in gallbladder contraction

(Thomson 1975). Since 1966 several peptide hormones and neuropeptides that effect gallbladder function have been identified. However, it seems that CCK remains the most potent of them in the regulation of the gallbladder.

Other peptide hormones, which have a known effect on gallbladder function are gastrin, secretin, motilin, pancreatic polypeptide (PP) and peptide YY (PYY). Gastrin causes gallbladder contraction in some species, but in man it is less potent (Valenzuela et al. 1976, Cantor et al. 1986). Secretin is released from the upper gut by acids and has been shown to potentiate the action of CCK on gallbladder contraction (Ryan and Cohen 1976). Also motilin is a direct stimulant of gallbladder contraction, but the influence of motilin on gallbladder contraction is weaker than that of CCK (Takahashi et al. 1982). PP and PYY have been shown to play a role in the regulation of gallbladder filling (Conter et al. 1987).

Neuropeptides that are known to effect gallbladder function are substance P, vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP) and somatostatin. Substance P stimulates gallbladder contraction, but it is less potent than CCK (Lonovics et al. 1985). VIP and CGRP inhibit the contractile response of the gallbladder to CCK (Ryan et al. 1977, Lonovics et al. 1979, Strah et al. 1986, Hashimoto et al. 1988) and somatostatin plays a role in the regulation of gallbladder filling (Fisher et al. 1987). The role of galanin and peptide histidine-isoleucine (PHI) are still unclear, but immunoreactivity for these peptide hormones has been found in the pig gallbladder (Sand et al. 1993).

2.3.1 Cholecystokinin

Since Jorpes and Mutt identified CCK as a linear polypeptide containing 33 amino acids (Jorpes and Mutt 1966), several molecular forms of CCK (CCK-8, CCK-39, CCK-58, CCK-83) have been isolated and purified (Rehfeld 1978, Kothary et al. 1983, Eberlein et al. 1987, Eberlein et al. 1992). CCK is released from the duodenum by luminal acid and nutrients, in particular fat and amino acids (Thompson et al. 1975). It has been shown that CCK-8 maintains nearly all of its systemic activity on its first passage through the liver and retains its capability for stimulating gallbladder contraction and pancreatic enzyme secretion (Sakamoto et al. 1985). In addition, the bigger molecular forms of CCK have been suspected of being responsible for gallbladder contraction (Eysselein et al. 1987, Reeve et al. 1996), but they may only be active during the first pass of portal circulation. The half life of CCK in the plasma is about 2.5 minutes and the kidney is mainly responsible for its uptake from the systemic circulation (Thompson et al. 1975).

The effects of CCK on gallbladder contraction are mediated by two pathways: (1) hormonally from the endocrine cells by circulation (Lilja et al. 1982) and (2) neurally by cholinergic vagal neurones (Liedberg 1969). It has been shown that the degree of gallbladder contraction caused by infusion of CCK-8 is directly proportional to the concentration of CCK detected in the plasma by radioimmunoassay (Lilja et al. 1982). Also, the CCK receptors of gallbladder smooth muscle have a high sensitivity to the C-terminal residue of CCK (Williams and Bailey 1986). The presence of pancreatic enzymes in the duodenum stimulated by CCK further inhibit CCK release (Owyang et al. 1986, Fölsch et al. 1987).

2.4 Neural and humoral control of Sphincter of Oddi

Histochemical studies have shown, that the SO has both adrenergic and cholinergic innervation in the cat and dog (Kyösola and Rechardt 1973, Kyösola 1974). Adrenergic stimulation causes the contraction of the SO (Grace et al. 1990), but the function of the vagus remains controversial. Although truncal vagotomy has been shown to increase the resistance of bile flow through the SO, other studies have failed to show any effect of vagal stimulation on SO contraction (Tansey et al. 1974, Pitt et al. 1982).

The function of the SO is also regulated by several peptides, which affect the smooth muscle of the SO directly and also via a neural pathway (Grace et al. 1990). CCK has been shown to relax the SO in humans, cats, dogs and pigs (Geenen et al. 1980, Behar and Biancani 1980, Grace et al. 1990).

While the CCK -induced SO relaxation was not antagonised by either adrenergic or cholinergic blockade, it has been suggested that the function of SO is also mediated by non-cholinergic, non-adrenergic neurones (Behar and Biancani 1980). Gastrin, secretin and glucagon relax or potentiate the relaxation of the SO induced by CCK, but the effect of these is weaker than the effect of CCK (Grace et al. 1990). Motilin has been shown to regulate the cyclic activity of the SO during the interdigestive period via opiate, serotoninergic and cholinergic neurones (Müller et al. 1987, Behar and Biancani 1988). The effects of VIP, neuropeptide Y, galanin, PHI and CGRP on the biliary motility remain unclear, but a strong immunoreactivity for them has been found in the nerves of the SO (Sand et al. 1993).

3 Pancreatitis

3.1 Pathogenesis in different etiologies

3.1.1 Alcohol induced acute pancreatitis

In Finland, alcohol accounted for only 10-20 % of the etiology of acute pancreatitis up to the seventies (Salmenkivi and Asp 1972), while nowadays it is the main cause of acute pancreatitis accounting for 70 % of cases (Jaakkola and Nordback 1993). The incidence of acute pancreatitis seems to correlate with alcohol consumption in the country (Jaakkola and Nordback 1993). Individually, the amount of recently consumed alcohol correlates with the severity of the first episode of acute pancreatitis, but not of recurrent alcoholic acute pancreatitis (Jaakkola et al. 1994). However, only 5 % of alcoholics develop overt clinical evidence of acute pancreatitis (Wilson et al. 1989, Singh and Simsek 1990). Numerous investigators have attempted to account for individual susceptibility by studying associations between alcoholic pancreatitis and potential risk factors. Whilst the role of genetic factors, dietary factors and smoking remains controversial (Haber et al. 1995, Whitcomb 2000), hyperlipidemia has a demonstrated association with acute pancreatitis (Cameron et al. 1971). It has been proposed that pancreatic lipase liberates toxic concentrations of free fatty acids that damage the capillary membranes (Havel 1969). Acetaldehyde, a metabolite of ethanol, can induce acute pancreatitis in isolated canine pancreas preparation (Nordback et al. 1991). This model of acute pancreatitis is mediated by oxygen-derived free radicals. Whether the initial injury is in the capillaries, acinar cells or pancreatic duct, remained obscure. Due to the lack of a good experimental alcoholic pancreatitis model, the pathogenesis remains unknown.

3.1.2 Gallstone disease induced acute pancreatitis

Although in most cases the episode of gallstone pancreatitis is mild, about 20 % of patients develop a severe disease with high morbidity rate (Bittner et al. 1987, Bradley and Allen 1991). It is well established that pancreatitis recurs in about 30 % of the cases if the gallstones are not removed (Paloyan et al. 1975). In gallstone pancreatitis the gallstones are migrating from the CBD through the ampulla into the duodenum (Acosta and Ledesma 1974). Most stones originate from the gallbladder, in a minority of cases the stones are primarily formed in the common bile duct (Kelly 1980).

3.1.2.1 Pathogenetic theories

In 1901 Opie reported a case where an impacted stone in the ampulla of Vateri resulted in hemorrhagic pancreatitis (Opie 1901). Since then the mechanism that triggers acute pancreatitis has been explained by three main theories: (1) the common channel theory suggests that a stone causes distal obstruction of the common channel allowing bile reflux into the PD (Opie 1901, Acosta and Ledesma 1974, Kelly 1976, Acosta et al. 1980); (2) the pancreatic duct obstruction theory suggests that stone impaction in the ampulla of Vateri causes PD obstruction resulting in ductal hypertension and, thus, initiates pancreatitis (Lerch et al. 1993); (3) the duodenal reflux theory suggests that a stone passage may stretch the SO allowing the duodenal fluid to reflux into the PD (McCutcheon and Race 1962).

According to the common channel theory, an impacted stone causes the functional common channel between the CBD and PD allowing the biliopancreatic reflux (Opie 1901). The opposite arguments have been presented, because under normal circumstances the PD pressure is higher than BD pressure (Csendes et al. 1979, Hernandez et al. 1997). It is not known, whether biliopancreatic reflux takes place, but in theory the common channel obstruction equalizes the PD and BD pressures and in the case of gallbladder contraction BD pressure increases over the PD pressure and biliopancreatic reflux occurs. However, the role of the gallbladder in pancreatitis has never been studied before. Furthermore, some studies have shown that the biliary-pancreatic tract morphology is different in gallstone pancreatitis patients as compared to CBD stone patients without a history of pancreatitis favouring the biliopancreatic reflux theory (Taylor and Rimmer 1980, McMahon et al. 1981, Osborne et al. 1983, Armstrong and Taylor 1986). Furthermore, in experimental studies bile

salt solution injected into the CBD causes acute pancreatitis (Aho and Nevalainen 1980, Aho et al. 1980).

According to the second theory, the obstruction in the PD causes hypertension, ductal disruption and acinar cell injury. It has been shown in experimental studies that CBD ligation in opossum causes acute necrotizing pancreatitis, and that the severity of the pancreatitis episode correlates with the time of obstruction (Lerch et al. 1992, Lerch et al. 1993). So, it has been shown that both bile reflux and obstruction are capable of inducing experimental acute pancreatitis. The duodenal reflux theory is further undermined by the clinical evidence that only a minority of patients undergoing ERCP, endoscopic sphincterotomy (EST), pancreatic sphincterotomy or pancreatic stent placement develop acute pancreatitis.

3.1.2.2 Pancreatic enzyme activation

In normal physiological circumstances the pancreatic digestive enzymes are secreted as inactive proenzymes or zymogens that become activated by enterokinase only after reaching the duodenum (Steer 1995). In addition, inhibitors of proteases are synthetisized and transported along with the zymogens (Steer 1995). But in acute pancreatitis, the function of the acinar cells are disturbed and the activation of trypsin results in the activation of other digestive enzymes within the acinar cell (Steer 1995, Lerch et al. 1995). It is not clear, what exactly triggers this premature activation of the enzymes within the acinar cell, but in three models of experimental pancreatitis (induced by choline-deficient, ethionine-supplemented diet; cerulein; or obstruction) a phenomenon, called colocalization is observed (Gilliland and Steer 1980, Watanabe et al. 1984, Saluja et al. 1985, Saito et al. 1986, Saluja et al. 1987, Ohshio et al. 1989, Saluja et al. 1989). In colocalization, the normal separation of digestive zymogens from lysosomal enzymes remains defective in acute pancreatitis and, as a result, both zymogens and lysosomal enzymes are packaged into the condensing vacuoles (Steer 1995). In these intracellular vacuoles the lysosomal hydrolase cathepsin B can probably activate trypsinogen, which further activates the remaining zymogens (Figarella et al. 1988).

3.1.2.3 Role of CCK

Several experimental studies have shown that the CCK agonist cerulein can induce acute pancreatitis (Evander et al. 1981, Saluja et al. 1987, Figarella et al. 1988, Clemens et al. 1991, Nordback et al. 1991, Niederau et al. 1996, Plusczyk et al. 1997, Taniguchi et al. 1997). Furthermore, CCK receptor antagonists have been shown to beneficially affect the course of experimental acute pancreatitis (Niederau et al. 1986, Modlin et al. 1989, Larsen et al. 1991, Tani et al. 1993, Kobayashi et al. 1996, Taniguchi et al. 1997). Modern radioimmunoassay has made it possible to measure circulating plasma CCK levels (Liddle 1998). There appears to be only one previous report about human plasma CCK levels among acute pancreatitis patients (Shirohara and Otsuki 1997). They reported the plasma CCK levels in eight patients with acute gallstone pancreatitis, four patients with acute alcoholic pancreatitis and one patient with post-ERCP pancreatitis, and found the highest levels of CCK amongst the gallstone pancreatitis patients (Shirohara and Otsuki 1997). Large series and longitudinal observations of CCK in various pancreatitis etiologies are missing.

3.1.2.4 Role of microbes

In normal circumstances the biliary tree is thought to be sterile, but when CBD stones are present, bile is contaminated with bacteria in up to the 88 % of cases, although clinical infection is not present (Sand et al. 1992). Bacteria are seen by a scanning electron microscopy in 60 to 80 % of pigment and composite stones (Smith et al. 1989). The most common bacteria in the biliary tree are Eschericia coli, Streptococci and Clostridium perfringens (Sand et al. 1992). In mild edematous pancreatitis clinical infection is rare (Beger et al. 1986, Bradley 1989, Widdison and Karanjia 1993), but in necrotizing pancreatitis the necrosis is infected in 40 to 70 % of cases (Beger et al. 1986, Gerzof et al. 1987, Bassi et al. 1989).

It has been shown that non-infected bile salts can induce experimental acute pancreatitis (Aho and Nevalainen 1980). However, other studies have shown that the instillation of sterile bile into the PD under carefully controlled luminal pressure does not cause pancreatitis, whereas infected bile will induce acute pancreatitis (Amstrong et al. 1985, Arendt et al. 1999). Furthermore, antibiotics have been shown to increase survival rates in experimental acute pancreatitis (Araida et al. 1995). Inflammatory mediators, cytokines, have been shown to play a role in acute pancreatitis (Bhatia et al. 2000), and furthermore bacterial toxins may release cytokines from monocytes resulting in pancreatitis (Keynes 1987). In humans, the early endotoxemia frequently found in pancreatitis

(Kivilaakso et al. 1984) may also suggest the importance of bacteria early in the course. The role of bacteria in human gallstone pancreatitis, is, however extremely difficult to study.

3.1.3 ERCP induced acute pancreatitis

Hyperamylasemia follows ERCP in up to 60-70 % of patients, but clinical acute post-ERCP pancreatitis develops in only 5-7 % of patients (Sherman and Lehman 1991, Freeman et al. 1996). The potential mechanisms of pancreatic injury during ERCP may be the procedure-related mechanisms: (1) mechanical manipulation of papilla (Hamilton et al. 1983), (2) chemical nature of contrast media (Bub et al. 1983), (3) hydrostatic pressure (Roszler and Campbell 1985, Cunliffe et al. 1987), (4) enzymatic premature activation of proteolytic enzymes (Sherman and Lehman 1991), (5) microbiological (Keynes 1987) and (6) thermal injury (Sivak 1989). So-called patient-related risk factors include: (7) young age, (8) dysfunction of the SO and (9) a small BD diameter (Mehta et al. 1998). A large meta-analysis of the risk factors found significance in univariate analysis, five turned out to be independent also in multivariate analysis: suspected dysfunction of the SO, young age, precutting sphincterotomy, difficult cannulation and the number of pancreatic contrast injections (Freeman et al. 1996). Although, a microbiological mechanism for the induction of post-ERCP pancreatitis has been suspected before (Nordback and Airo 1988), there are no previous prospective studies on this topic.

3.2 Diagnostic criteria

3.2.1 Clinical signs, laboratory tests and radiology

According to the modern definition (Atlanta Symposium), acute pancreatitis is an acute inflammatory process of the pancreas, with variable involvement of other regional tissue or remote organ systems (Bradley 1992). Classically, an attack of acute pancreatitis is manifested by the sudden onset of severe constant abdominal pain radiating through to the back (Pollock 1959).
Acute pancreatitis is often accompanied by vomiting, fever, tachycardia, leukocytosis and elevated pancreatic enzyme levels in the blood and/or urine (Bradley 1992). Although, there is no gold standard for the diagnosis of acute pancreatitis, an increase in amylase activity by three fold over the upper normal range has been considered to have over 90 % specificity to detect pancreatitis (Lin et al. 1989). When comparing the total serum amylase activity with pancreas specific amylase activity

or lipase activity, the specificity to detect acute pancreatitis is similar (Lin et al. 1989). Trypsinogen-2 has also been shown to be a good marker of acute pancreatitis, but until recently the assays have not been available in most hospitals (Itkonen et al. 1990, Hedström et al. 1994). The recently developed rapid urinary trypsinogen-2 test can detect acute pancreatitis with a high probability and is especially able to exclude pancreatitis in a severely ill patient (Kemppainen et al. 1997, Kylänpää-Bäck et al. 2000).

When the clinical picture, the history of alcohol abuse or gallstone disease or other predisponding factors, and laboratory measurements are combined the specificity of the diagnosis of acute pancreatitis is over 95 % (Marshall 1993). Ultrasound is often needed in the assessment of the etiology of pancreatitis episode. In unclear cases, computed tomography (CT) can offer further information (Kivisaari et al. 1983), being a standard tool to diagnose acute necrotizing pancreatitis (Kemppainen et al. 1998).

3.2.2 Classification and severity

Acute pancreatitis can be divided morphologically into (1) edematous disease with interstitial pancreatic edema and limited peripancreatic fat necrosis, and (2) necrotizing disease with extensive parenchymal necrosis, areas of hemorrhage and extensive peripancreatic fat necrosis (Bradley 1992).

Two main scoring systems (Ranson and Glasgow) have been used over the last 20 years in attempting to predict the clinical severity of the disease in the early phase (Ranson and Pasternack 1977, Leese and Shaw 1988). According to them the severity of an acute pancreatitis episode can be predicted in the early phase of the disease with 70-80 % specificity. The development of the disease in intensive care units can be followed by the APACHE-II scoring system (Larvin and McMahon 1989). However, in clinical practice all these scoring systems are rather cumbersome and not so widely used in Finland. In the classification of post-ERCP pancreatitis, a new consensus criteria, based on the hospital stay and the need for invasive procedures have been recently described (Cotton et al. 1991).

Serum amylase activity does not correlate with the severity of pancreatitis episode, and so there is no value in monitoring amylase activity during the episode of acute pancreatitis (Nordback 1985). C-reactive protein (CRP) is a very valuable test in the assessment of the severity of acute pancreatitis episode and especially in the monitoring of the course (Mayer et al. 1984, Puolakkainen et al. 1987). The determination of phospholipase A2, trypsinogen-2 and pancreatitis associated

protein (PAP) are other attempted methods in the assessment of the severity (Puolakkainen 1987, Sainio et al. 1996, Kemppainen et al. 1996).

Contrast-enhanced CT has been shown to be a reliable method for determining pancreatic necrosis (Kivisaari et al. 1983), and performed early in the course of acute pancreatitis can accurately identify patients with severe pancreatitis (Balthazar et al. 1990). The severity of the inflammatory process and the extent of necrosis assessed by contrast-enhanced CT correlate with the morbidity and mortality of the pancreatitis episode (Balthazar et al. 1990). The results of contrast-enhanced magnetic resonance imaging (MRI) in the detection of severe acute pancreatitis are also promising, but the drainage procedures still remain a challenge for MRI (Piiroinen et al. 2000).

3.3 Complications

Acute pancreatitis may develop several local and systemic complications (Pitchumoni et al. 1988). Early complications of acute pancreatitis consist of septic complications, renal dysfunction, lung dysfunction and haemorrhage. Furthermore, acute pancreatitis may develop chronic pancreatitis, which may lead to exocrine and endocrine pancreatic insufficiency. In addition to systemic complications, chronic pancreatitis may also develop local complications, for instance pancreatic pseudocysts. Although, pseudocysts are also seen in acute phase of pancreatitis episode, they may be formed later and lead to obstructive jaundice, duodenal compression or haemorrhage (Kiviluoto et al. 1989). The highest morbidity and mortality rate is, however, connected with those early septic or infection complications (Beger et al. 1986).

3.3.1 Infected pancreatic necrosis

Infection of the pancreatic necrosis correlates with the duration of the disease and the extent of necrosis, and is the single most common cause of death among the patients with necrotizing pancreatitis (Beger et al. 1986). The bacterial spectrum consists primarily of gram-negative bacteria, but in some cases also of Staphylococci, Streptococci, Enterococci, anaerobes and fungi (Beger et al. 1986, Gerzof et al. 1987, Bassi et al. 1989). The infection of necrotizing pancreatitis occurs in 24 % of patients after the first week from the onset of symptoms and in 71 % of patients after the third week (Beger 1986, Bassi et al. 1989, Widdison and Karanjia 1993).

Earlier studies failed to show any benefit of antibiotic prophylaxis in patients with acute pancreatitis (Howes et al. 1975). More recently, it has been shown that antibiotic regimens have big differences in pancreatic penetration (Büchler et al. 1992). Seven recently reported randomized studies suggest a beneficial role of antibiotic prophylaxis in patients with necrotizing pancreatitis, especially on septic complications (Pederzoli et al. 1993, Sainio et al. 1995, Luiten et al. 1995, Delcenserie et al. 1996, Schwarz et al. 1997, Bassi et al. 1998, Nordback et al. 2000).

Experimental studies have shown that bacteria may reach the pancreas via a number of different routes, such as the transcolonic route, via the main PD or via the circulation (Tarpila et al. 1988, Widdison et al. 1994). The mechanism in humans is unclear, although translocation from the colon has been strongly suggested (Wells et al. 1988). However, anaerobic bacteria that predominate in the colon, have been isolated only from 15 % of patients with pancreatic infection, whereas the proportion of the different species of bacteria causing such infections more closely resembles that

found in the bile (Keighley 1982). Surprisingly, the etiology of pancreatitis in studies with pancreatic infection has seldom been given extra attention.

AIMS OF THE STUDY

The aims of the present study were to investigate the role of gallbladder, CCK and microbes in gallstone and post-ERCP pancreatitis.

The specific aims were to study:

1 The association of the gallbladder with the severity of gallstone pancreatitis (I), and post-ERCP pancreatitis (V).

2 CCK in acute pancreatitis of different etiologies: post-ERCP pancreatitis (II), gallstone pancreatitis (III) and alcoholic pancreatitis (III).

3 The microbes of pancreatic necrosis in gallstone and alcoholic pancreatitis (IV).

4 The effect of pre-ERCP antibiotic prophylaxis (cephtazidime) on the post-ERCP pancreatitis (V).

PATIENTS AND METHODS

1 Study I – The role of the gallbladder in gallstone and post-ERCP pancreatitis

Altogether 297 consecutive patients with acute gallstone pancreatitis, treated between 1972-1992 at Tampere University Hospital, were included in this retrospective study. The patients with simultaneous acute cholecystitis or acute cholangitis were not included. Also 31 patients were excluded because of incomplete data.

Of the 266 included patients; 121 were males and 145 females with the median age of 55 (range 15-92) years. The diagnosis of gallstone pancreatitis was based on the clinical picture and an increased amylase activity by three fold or higher over the upper normal range. For the last 7 years of the study serum CRP level was also included. The gallstone etiology was based on positive cholecystography, ultrasonography, iv cholangiography, ERCP, surgery or autopsy. Patients were divided into two groups: A) those patients with a gallbladder in situ at the time of acute pancreatitis episode (n=234), 109 males and 125 females with a median age of 60 (range 21-92) years and B) those patients who had previously undergone cholecystectomy (n=32), 12 males and 20 females with a median age of 50 (range 15-87) years. Interventions, either open surgery or ERCP were performed in 44 % of patients in group A and 40 % of patients in group B.

We assessed both the severity and the complication rate between the study groups. We also investigated the effect of age on the severity of pancreatitis episode, because the study groups differed from each other by age. The severity of the pancreatitis episode was assessed by using the Glasgow criteria, CRP, development of necrotizing disease and mortality.

The presence of gallbladder was also studied in the post-ERCP pancreatitis material (see Study V).

2 Studies II and III - CCK in acute pancreatitis of different etiologies

In studies II and III we investigated circulating fasting plasma CCK levels in patients with pancreatitis of different etiologies.

We prospectively collected plasma samples in Tampere University Hospital from 342 patients who underwent ERCP and chose from those: 1) patients who had post-ERCP pancreatitis (n=23), 2) patients who had post-ERCP hyperamylasemia (AMYL > 600 IU/L) without pancreatitis (n=5), 3) patients who had post-ERCP abdominal pain without hyperamylasemia (n=18) and 4) randomly selected 43 patients with uneventful post-ERCP period. Plasma samples were collected in the morning of ERCP, 4 to 8 hours, 10 to 16 hours, and 24 hours after completing the ERCP. Furthermore, we collected plasma samples in Tampere and Helsinki University Hospitals for further groups: 5) patients with acute alcoholic pancreatitis (n=35), 6) patients with acute gallstone pancreatitis (n=27), 7) patients with non-pancreatic acute abdominal pain (n=34) and for the control group 8) healthy adult volunteers (n=43). In groups 5 to 7, plasma samples were taken on admission to the hospital. The mean time from the beginning of symptoms to the plasma sampling was 31 hours among alcoholic pancreatitis patients (group 5) and 25 hours among gallstone pancreatitis patients (group 6). All the patients in the groups 1 to 8 had fasted for at least 6 hours before each sample. Samples were stored at -70°C for later assays of CCK, AMYL and CRP. We also determined CCK levels serially in 20 patients during a course of acute pancreatitis.

The diagnosis of acute pancreatitis in groups 1, 5 and 6 was based on the clinical picture and on an increased serum amylase activity by three fold or higher over the upper normal range. The etiology of pancreatitis in groups 5 and 6 was verified by ultrasonography or CT. The severity of the pancreatitis episode was assessed by using Ranson`s prognostic criteria and the CRP level. The clinical data of the patients in study groups 1 to 4 is seen in Study II, Table 1 and in groups 5 to 8 in Study III, Table 1.

2.1 Assay procedures

CCK concentrations were determined by a specific and sensitive radioimmunoassay using CCK antiserum (Euro-Diagnostica, Malmö, Sweden). The normal fasting level of CCK is < 1.12 pmol/L according to the manufacturer. Amylase activity was measured with an enzymatic calorimetric test, using 2-chloro-4nitro-phenyl-β-D-malto-hepatoside (4 mmol/L) as a substrate (Raucher and Gerber 1989). Serum amylase activity of less than 300 IU/L was considered normal. CRP was determined by using an immunoturbidometric method with antiserum and standards from Orion Diagnostica, Finland (Harmoinen 1985). The upper reference limit was 10 mg/L.

3 Study IV – The microbes of pancreatic necrosis in gallstone and alcoholic pancreatitis

Between 1972-1992 a total of 1854 cases of acute pancreatitis were treated in Tampere University Hospital. In this study we retrospectively investigated the microbes of pancreatic necrosis in gallstone and alcoholic pancreatitis patients and included all operatively treated, 128 (7%) patients. Fifty-eight patients were excluded because of incomplete data, simultaneous acute cholecystitis or those who had cholecystolithiasis and alcoholism at the same time. The 70 included patients were divided into two groups: A) patients with recent alcoholism without gallstones (n=47), and B) patients with gallstones without the history of previous alcohol misuse (n=23). There were 42 males and 5 females with median age of 42 (range 21-65) years in the alcoholic pancreatitis group; 12 males and 11 females with median age of 43 (range 23-65) years in the gallstone pancreatitis group. According to the Glasgow criteria, 81 % of the patients in the alcoholic pancreatitis group and 83 % of the patients in the gallstone pancreatitis group had a severe episode of acute pancreatitis. In all the patients included the necrotizing form of pancreatitis was confirmed at the operation. The extent of necrosis, assessed in the operation, did not differ between the study groups (Study IV, Table 1).
The microbes of the pancreatic necrosis were isolated by aerobic and anaerobic cultures and cultures for fungi. The median time from the first symptoms to the operation was 13 (95% CI, 7-20) days in the alcoholic pancreatitis group and 7 (4-11) days in the gallstone pancreatitis group. Antibiotic therapy was given preoperatively to 74 % of the patients in the alcoholic pancreatitis group and 83 % of the patients in the gallstone pancreatitis group. There was no difference in the duration of the preoperative antibiotic therapy, nor the spectrum of antibiotics that were used between the study groups.

4 Study V – The effect of antibiotic prophylaxis on post-ERCP pancreatitis

Between 1993 and 1996 a total of 953 ERCP examinations were made in Tampere University Hospital. During that time a total of 321 patients were prospectively randomized on admission for ERCP into two groups: 1) prophylaxis group (n=161), 2 g cephtazidime intravenously 30 minutes before ERCP and 2) control group (n=160), no antibiotic. All patients, who had not taken any antibiotics in the preceding week were included. Patients with an allergy to cephalosporins, with immune deficiency or any other condition requiring mandatory antibiotic prophylaxis, patients with severe jaundice and pregnant patients were excluded. Antibiotic treatment was continued after the examination (1 g x 2 cephtazidime) in six patients, if the biliary obstruction could not be removed. Therefore, from the final analysis six out of the 231 randomized patients were excluded. The final study population constituted 155 patients in the prophylaxis group and 160 patients in the control group. There was no difference in sex, age, underlying disease, degree of pre-ERCP biliary obstruction, interventions or ERCP diagnosis between the study groups (Study V, Table 1).

The primary endpoint was the incidence of clinical pancreatitis and cholangitis during the seven days after the ERCP. Secondarily, we also studied the prevalence of hyperamylasemia (≥ 900 IU/L) and the presence of gallbladder.

Serum samples were collected in all patients just before the ERCP, 4-8 hours and 18-24 hours after the ERCP to determine AMYL, LEUK, CRP, ALAT, AFOS, and BIL. Additional samples were taken whenever clinically indicated.

The diagnosis of post-ERCP pancreatitis was based on the clinical picture, an increased AMYL (≥ 900 IU/L), an increased CRP, and the absence of symptoms of cholangitis. The diagnosis of cholangitis was based on a continuously rising or septic fever, an increased CRP level, leukocytosis and increasing liver function values.

5 Statistics

Fisher`s exact test (Studies I, III-V), Mann-Whitney U-test (Studies I, III, IV, V), forward stepwise multiple logistic regression analysis (Studies I, IV, V), Kruskal-Wallis nonparametric analysis of variance and Spearman correlation coefficients (Study II) were used to assess the significance of differences between the groups. Differences of $p < 0.05$ were considered significant.

The values in studies II and III were given as the mean and standard error of mean (SEM) or as the median and 95 % confidence interval (CI).

6 Ethical aspects

The study was conducted in accordance with the Helsinki Declaration and the study protocols were approved by the Ethical Committee of Tampere University Hospital.

RESULTS

1 Study I – The role of the gallbladder in gallstone and post-ERCP pancreatitis

The patients with gallbladder in situ at the time of acute pancreatitis episode had according to positive Glasgow criteria (≥ 3) and to the hospital mortality rate (Study I, Table 1) significantly more severe disease compared to the patients who had previously undergone cholecystectomy. The hospital mortality was consistently different between the groups when studied in four periods (1972-1976, 1977-1981, 1982-1986, 1987-1992), without a tendency to decline with time. Also, twice as many patients had high admission CRP (> 150 mg/L) concentration in the gallbladder in situ -group (Study I, Table 1). Although the patients with their gallbladder in situ at the time of pancreatitis episode were 10 years older than the non-gallbladder -group patients, the older age did not increase the risk of severe disease with high CRP (OR=1.00), whereas having a gallbladder in situ did increase the risk of severe disease by over two-fold (OR=2.57). Fourteen per cent of patients with a gallbladder had a necrotizing disease compared to the six per cent of the patients who had previously undergone cholecystectomy, but this difference was not statistically significant.

Eighty-one patients (30%) developed complications, significantly more frequently in the gallbladder in situ -group (Study I, Table 2). The most common complications were sepsis, shock, renal -and pulmonary insufficiencies, however there were no significant differences in the levels of the separate complications between the study groups (Study I, Table 2). During 1982-1992, when abscesses were diagnosed by CT, significantly more abscesses were diagnosed in the gallbladder in situ -group (5/114 vs 0/14, p=0.05).

In post-ERCP pancreatitis (Study V), the presence of the gallbladder did not effect on the development of the disease.

Severity and complications in Group A and Group B

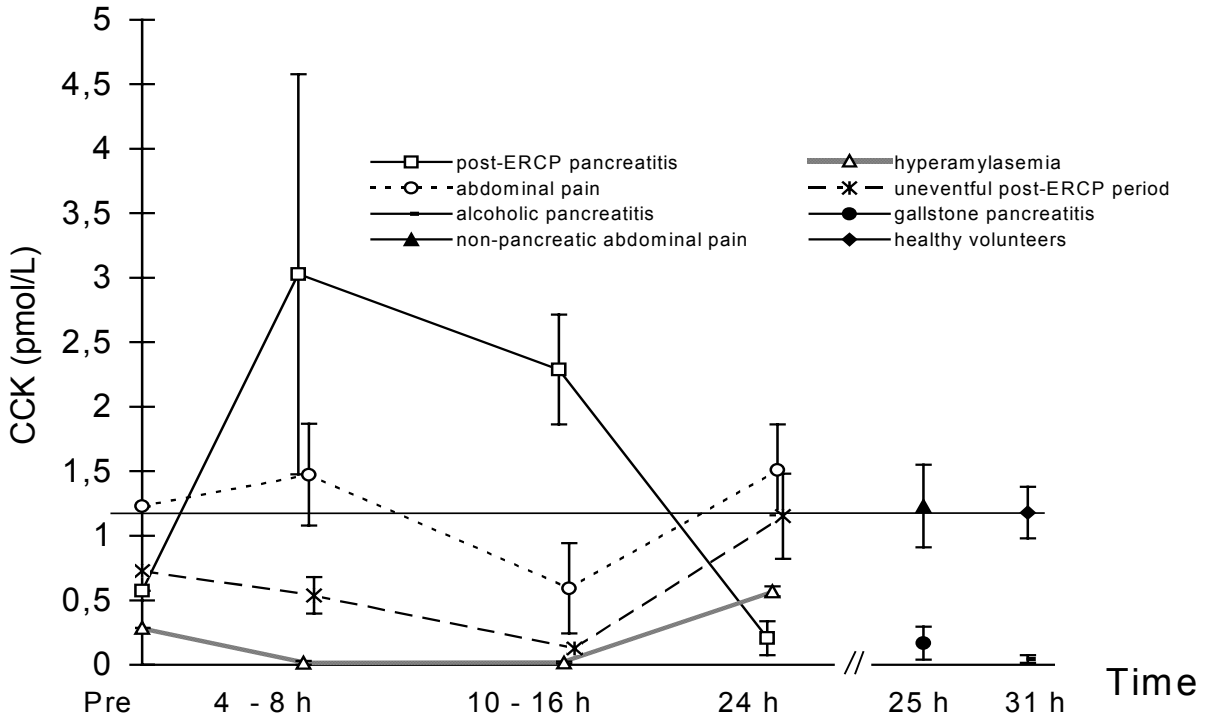
	Group A, n = 234	Group B, n = 32	p*
Positive Glasgow criteria (≥3)	66/210 (31%)	4/29 (14%)	0.04
CRP > 150 mg/L	50/102 (49%)	3/12 (25%)	0.10
Necrotizing pancreatitis	33/234 (14%)	2/32 (6%)	0.17
Mortality	40/234 (17%)	1/32 (3%)	0.03
Patients with complications	77/234 (33%)	4/32 (13%)	0.01

* Fisher`s exact test

2 Studies II and III - CCK in acute pancreatitis of different etiologies

In the post-ERCP pancreatitis patients, plasma CCK levels increased by five fold early in the course of the disease and then gradually decreased to nearly unmeasurable levels within 24 hours (Figure 1). At that same time point, about one day from the beginning of symptoms, similar low CCK levels were also found in the alcoholic (mean ± SEM, 0.04 ± 0.03 pmol/L) and the gallstone (0.17 ± 0.13 pmol/L) pancreatitis patients (Figure 1). In the hyperamylasemia patients and in the patients with an uneventful post-ERCP period, plasma CCK levels temporarily decreased after the ERCP and normalized within 24 hours. In the patients with post-ERCP abdominal pain without pancreatitis, plasma CCK levels remained at the same levels as before ERCP and those levels were similar to the CCK levels in patients with non-pancreatic acute abdominal pain (1.23 ± 0.32 pmol/L) or in healthy volunteers (1.18 ± 0.20 pmol/L) (Figure 1).

Figure 1. Mean CCK in the study groups



Of the 23 post-ERCP pancreatitis patients, three developed a severe episode of pancreatitis (≥ 3 Ranson signs, necrosis in CT) and two of these three patients had higher CCK levels early in the course than the mean CCK level of all post-ERCP pancreatitis patients. Seven patients (20%) with alcoholic pancreatitis and five patients (19 %) with gallstone pancreatitis developed severe episodes of pancreatitis and all of them had very low CCK levels on admission. CCK levels also remained low until the patient was recovering well and had started an oral diet (Study III, Figure 2). The median CCK level remained low until the second the last measurement [median and 95 % CI; 0.18 (0.08, 0.65) pmol/L, at median 4.0 (3.64, 6.87) days after the onset of symptoms], compared to last measurement close to discharge [1.30 (1.08, 2.75) pmol/L, at median 7.5 (6.08, 10.72) days after the onset of symptoms], p= 0.001. By that time the serum CRP level had decreased from the highest level [123 (98, 143) mg/L] to the lower level [57 (44, 67) mg/L].

Interestingly, those post-ERCP pancreatitis patients who had the gallbladder in situ had the highest mean CCK levels during 4-8 hours after the ERCP compared to the patients who had previously undergone cholecystectomy (mean±SEM, 4.85±2.34 vs 0.12±0.12 pmol/L, p=0.005). In patients with non-pancreatic acute abdominal pain we found the highest CCK levels with bile duct or gallbladder stones, but also in patients with hepatic cirrhosis and in one patient with intestinal occlusion (Study III, Table 3).

3 Study IV – The microbes of pancreatic necrosis in gallstone and alcoholic pancreatitis

Microbes were isolated more often in cultures from pancreatic necrosis in the gallstone pancreatitis patients compared to the alcoholic pancreatitis patients (15/47 vs 17/23, p=0.001) (Study IV, Table 2). The most common microbes in both study groups were Eschericia coli, Staphylococci and Candida species, although Staphylococci were cultured three times more often from the pancreatic necrosis in the alcoholic pancreatitis patients (Study IV, Table 2). Four alcoholic pancreatitis patients and five gallstone pancreatitis patients had a polymicrobial infection. Gram-negative bacteria were most common in the gallstone pancreatitis patients (gallstone vs. alcoholic pancreatitis, 11/17 vs 6/15, p=0.15), whereas Gram-positive bacteria were most common in the alcoholic pancreatitis patients (9/15 vs 6/17, p=0.15), although the differences did not reach statistical significance (Study IV, Table 3). When the operation was done during the first week from the beginning of symptoms, Gram-negative bacteria were isolated more often from the necrosis of the gallstone pancreatitis patients (8/10 vs 1/5, p=0.04), and when the operation was done later in the course, Gram-positive bacteria were isolated twice as often in the alcoholic pancreatitis patients as in the gallstone pancreatitis patients (7/10 vs 2/7, p=0.12) (Study IV, Table 4).

Microbiology of pancreatic necrosis in gallstone and alcoholic pancreatitis

	Gallstone, n = 23		Alcoholic, n = 47		p*	
Positive cultures	17/23 (74%)		15/47 (32%)		0.001	
	1-7 d	>7 d	1-7 d	>7 d	1-7 d	>7 d
Gram-negative	8/10 (80%)	2/7 (29%)	1/5 (20%)	5/10 (50%)	0.04	0.35
Gram-positive	4/10 (40%)	2/7 (29%)	2/5 (40%)	7/10 (70%)	0.71	0.12

* Fisher´s exact test

In a multivariate analysis of the etiology, severity of pancreatitis episode, age, time of the first symptoms to the operation, and sex, we found that only the gallstone etiology (OR 5.5, 95 % CI 0.59-52.1) and the severity of pancreatitis (7.7, 0.55-108.1) were independent risk factors in developing contamination with Gram-negative bacteria.

4 Study V – The effect of antibiotic prophylaxis on post-ERCP pancreatitis

The control group patients had both post-ERCP pancreatitis (15/160 vs 4/155, p=0.009) and cholangitis (7/160 vs 0/155, p=0.009) more often than the prophylaxis group patients (Study V, Table 2). The post-ERCP levels of AMYL, LEUK and CRP, and both pre -and post-ERCP levels of ALAT, AFOS and BIL are presented in the Table 1 for the patients who developed complications.

Table 1. The mean and 95 % confidence interval (95 % CI) of the laboratory values in the patients with complications

	Post-ERCP pancreatitis n=19 (6 %)	Cholangitis n=7 (2.2 %)
AMYL (IU/L)	4943 (2257-7628)	418 (62-607)
LEUK (L)	15 (9-25) x 10^{-3}	14 (9.5-24) x 10^{-3}
CRP (mg/L)	111(70-152)	68 (20-115)
ALAT (IU/L)		
pre	49 (17-81)	41 (29-64)
post	41 (23-58)	90 (35-145)
AFOS (IU/L)		
pre	184 (151-217)	202 (79-324)
post	189 (158-221)	340 (203-477)
BIL (IU/L)		
pre	24 (7-40)	10 (5-25)
post	23 (8-38)	74 (7-150)

In patients with post-ERCP pancreatitis the post-ERCP levels of ALAT, AFOS and BIL did not differ from the levels observed before the ERCP, whereas in patients with cholangitis the levels were increased (Table 1).

Three pancreatitis patients developed a severe episode according to the Ranson`s prognostic criteria, one patient in the prophylaxis group and two patients in the control group (NS difference). If we use the consensus classification criteria recently made (Cotton et al. 1991), ten patients had a mild disease, nine patients had a moderate disease and none had a severe disease. CT were undertaken in two out of the three patients with severe pancreatitis according to the Ranson criteria, and the findings were fluid collections and edema. All post-ERCP pancreatitis patients were treated conservatively and none of them died. None of the patients needed to undergo fine needle aspiration for microbiological analysis of the necrosis. Three out of seven cholangitis patients had positive

blood cultures, Eschericia coli was found in two patients and Streptococci in one patient. All post-ERCP cholangitis patients responded to antibiotic therapy.

Four patients in the control group and one patient in the prophylaxis group also developed other infectious complications: two cholecystitis, one pneumonia and one urinary infection in the control group, and one cholecystitis in the prophylaxis group. Two patients developed post-procedure bleeding, one in each study group, and one patient in the prophylaxis group developed duodenal perforation. Patients with cholecystitis underwent operation, whereas other patients with complications were treated conservatively.

In a multivariate analysis of the risk factors previously considered important (Freeman et al. 1996), in conjunction with antibiotic prophylaxis, two factors that increase the risk of post-ERCP pancreatitis became independently significant: the lack of antibiotic prophylaxis (OR=6.63, p=0.03) and sphincterotomy (OR= 5.60, p=0.05) (Study V, Table 3).

Although 24 % of the patients developed hyperamylasemia (> 900 IU/L), only 44 % of the hyperamylasemia patients in the prophylaxis group developed acute pancreatitis as diagnosed here, whereas all of the hyperamylasemia patients in the control group developed acute pancreatitis (p=0.003) (Study V, Table 4).

Post-ERCP pancreatitis and the risk factors

	Prophylaxis group	Control group	p*		
Post-ERCP pancreatitis	4/155 (2.6%)	15/160 (9.4%)	0.009		
Risk factors			p**	OR**	95%CI
Lack of prophylaxis			0.03	6.63	1.2-35.7
Sphincterotomy			0.05	5.60	1.0-30.5

* Fisher`s exact test, ** forward stepwise multiple regression

DISCUSSION

1 The role of gallbladder in acute pancreatitis

Since Opie described the case in 1901, where an impacted stone in the ampulla of Vateri triggered an episode of acute pancreatitis, a number of experimental studies on the pathogenetic mechanisms of gallstone pancreatitis have been conducted. It has been shown, that both ductal obstruction (Lerch et al. 1993) and biliopancreatic reflux (Aho and Nevalainen 1980) may induce experimental acute pancreatitis. Lerch and colleagues found that separately ligated PD or BD caused similar acinar cell necrosis as the ligation of CBD, indicating that neither biliary nor duodenal reflux is needed for the induction of acute necrotizing pancreatitis. They concluded that obstruction is the key phenomenon that triggers experimental pancreatitis and the degree of acinar cell necrosis increases in relation to the time of the obstruction in the opossum. In man, the impaction of a stone is usually temporary, 48 hours from the beginning of gallstone pancreatitis episodes only 5 % of the patients still have impacted stones (Paloyan et al. 1975, Ranson 1979). According to these experimental and clinical data, it seems that the temporary obstruction and the consequent increase in the PD pressure may be one important trigger in the induction of acute gallstone pancreatitis. Another possibility is the biliopancreatic reflux. Experimental studies have also shown that bile salt injected into the PD can induce at least edematous acute pancreatitis with acinar cell necrosis in up to 10 % of the pancreas (Aho et al. 1983). However, other studies have shown that sterile bile injected into the PD under carefully controlled luminal pressure does not cause pancreatitis, whereas bile infected with Eschericia coli can induce acute pancreatitis (Amstrong et al. 1985, Arendt et al. 1999).

In normal circumstances the CBD pressure is under 7 mmHg and the PD pressure is under 12 mmHg (Carr-Locke and Gregg 1981). In gallstone pancreatitis, in the case of a common channel, the distal obstruction theoretically equalizes the pressures between the PD and the CBD. Then, in the case of forced gallbladder contraction bile reflux might take place. This possible mechanism in human acute gallstone pancreatitis is very difficult to prove, but some anatomical facts of the biliary tree suggest this possibility. First, the common channel is found in up to 70 % of the cases among gallstone pancreatitis patients (Uomo et al. 1993), and secondly, a high junction of CBD and PD have an association with an increased incidence of acute pancreatitis (Misra and Dwivedi 1990).

The present study showed that patients with the gallbladder in situ at the time of a gallstone pancreatitis episode developed more severe disease than patients who had previously undergone

cholecystectomy (when analysed according to the Glasgow criteria, the development of complications and mortality). During the last seven years of the study, when CRP levels were determined, we also found high CRP levels (> 150 mg/L) twice as often in patients with a gallbladder in situ as in patients who had previously undergone cholecystectomy. Furthermore, when the study period was divided into four periods (1972-1976, 1977-1981, 1982-1986, 1987-1992), the result did not change.

Obviously, because of retrospective nature of this study, the reliability of the pancreatitis diagnosis could be questioned. To increase the reliability, we included only those patients with a typical clinical picture, increased CRP level (during the last 7 years) and serum amylase activity of three fold or more over the upper normal range. An increase in amylase activity by three fold has been considered to have over 90 % specificity to detect pancreatitis in the early phase of the disease (Lin et al. 1989). Furthermore, to increase the reliability of determining the gallstone etiology, we excluded patients with recent alcoholism, hyperlipidemia and those with later diagnosed pancreatic neoplasm, even when gallstones were present.

The association between the presence of a gallbladder and the severity of the pancreatitis episode support the hypothesis that biliopancreatic reflux might take place in at least some gallstone pancreatitis patients. Conversely, in post-ERCP pancreatitis, the presence of a gallbladder was not associated with the development or the severity of pancreatitis. This may suggest that in post-ERCP pancreatitis biliopancreatic reflux through a common channel is not important in the pathogenesis.

2 The role of CCK in acute pancreatitis

Since the findings that CCK stimulates gallbladder contraction and pancreatic enzyme secretion (Thompson et al. 1975, Grace et al. 1990), its possible connections with pancreatitis have been suggested. Animal experiments have shown that a high dose of the CCK agonist cerulein, can induce acute pancreatitis, and the administration of exogenous CCK exacerbates the course of cerulein-induced pancreatitis (Evander et al. 1981). CCK receptor antagonists also have beneficial effects on the course of cerulein-induced pancreatitis, when given prophylactically or even therapeutically early in the course (Niederau et al. 1986, Nordback et al. 1991, Taniguchi et al. 1997). To further study the role of CCK in human acute pancreatitis serum measurements were performed.

In this study we prospectively determined fasting plasma CCK levels in post-ERCP pancreatitis, in gallstone pancreatitis and in alcoholic pancreatitis. We determined CCK levels before ERCP and for 24 hours after ERCP. Among the gallstone and alcoholic pancreatitis patients we determined CCK

levels on admission to the hospital and during the recovery process. To exclude the normal cyclic period of CCK release, we investigated only basal, fasting CCK levels. Our method was based on CCK-8 measurement, because this major form of CCK maintains nearly all its activity when passing through the liver and, thus, best reflects secretion of CCK into the circulation (Sakamoto et al. 1985). However, the circulating CCK levels may not fully reflect the local concentrations of CCK effecting in a paracrine way that may also be important.

To our knowledge there is only one previous report about human plasma CCK levels in acute pancreatitis (Shirohara and Otsuki 1997). They reported the peak CCK levels over a three-day period in one patient with post-ERCP pancreatitis, in eight patients with acute gallstone pancreatitis and in four patients with acute alcoholic pancreatitis, and found the highest CCK levels in gallstone pancreatitis patients. Interestingly, in the study by Shirohara and Otsuki, they found the highest CCK levels in patients with a mild course of the disease.

In the first hours we found a five fold increase in CCK levels in those patients who developed post-ERCP pancreatitis. This is a much larger and more prolonged increase than the post-prandial levels in normal subjects (Liddle et al. 1985). Thereafter, CCK levels gradually decreased to almost undetectable levels over a 24 hours period. By contrast, in other patients who underwent ERCP, CCK levels did not vary much and after 24 hours the levels were similar to the levels found before ERCP. None of the patients had a nasogastric tube, which theoretically could irritate the duodenum. Furthermore, there were no differences in medication or renal function between the study groups, which could effect CCK levels. One day after the beginning of the symptoms, the patients with gallstone or alcoholic pancreatitis had almost unmeasurable CCK levels, and the levels also remained low, independent of the etiology, until the patients were well recovered and had started an oral diet. We also found that patients with a gallbladder in situ had higher CCK levels than patients who had previously undergone cholecystectomy. CCK receptors are found in greater numbers in the muscle coat of the gallbladder than in other parts of the gut (Grider and Makhlouf 1987). It remains obscure, why the lack of this target organ was associated with increased CCK levels. Gallbladder – SO reflex connections have been described (Müller et al. 1984), so gallbladder – duodenal reflexes may also exist. These results are not in full agreement with those of Shirohara`s study. However, the lack of exact time point of each measurement in Shirohara`s study makes it possible that the patients with the highest CCK levels were actually already recovering from the pancreatitis episode, whereafter no discrepancy to the present study exists.

It is unclear, why the CCK levels are only elevated during the first hours (post-ERCP pancreatitis), and then after the first day from the beginning of symptoms the levels are almost unmeasurable

(post-ERCP, gallstone and alcoholic pancreatitis). One possible explanation for the early increase in CCK levels is that, patients who develop post-ERCP pancreatitis have more endoscopy or cannulation-induced irritation; resulting in an exaggerated CCK release. This CCK release might have an effect on the developing pancreatitis. Another possibility is that the CCK release is secondary. The presence of pancreatic enzymes in the duodenum inhibit CCK release (Fölsch et al. 1987). In pancreatitis the pancreas rapidly shuts down the synthesis and secretion of these enzymes (Iovanna et al 1995), therefore the CCK levels should increase, due to the lack of inhibitory pancreatic enzymes. The second notable finding was the decrease in CCK during later half of the first disease day. The reason also for this observation is unknown. One possible explanation is that the inflammation of the duodenum often seen in pancreatitis disturbs the feedback mechanism, which might actually be considered beneficial when the pancreas is not further stimulated during pancreatitis. Only obtaining serum samples from patients with gallstone pancreatitis at the beginning of symptoms, and the subsequent measurement of CCK could give further important information of the pathogenetic role of this peptide hormone in human acute gallstone pancreatitis. Interestingly, in experimental cerulein-induced acute pancreatitis, CCK receptor antagonists also have an ameliorative effect on the course of the disease only when given prophylactically or during the first hours of the induction of pancreatitis (Niederau et al. 1986, Modlin et al. 1989, Nordback et al. 1991, Larsen et al. 1991, Tani et al. 1993, Kobayashi et al. 1996, Taniguchi et al. 1997). The present study and the previous experimental studies do not exclude and possibly support the role of CCK early in the course of pancreatitis. Theoretically, taking into consideration the other data presented in this thesis which indicates the importance of the gallbladder in the determination of the severity of gallstone pancreatitis, early CCK increase might effect pancreatitis also via gallbladder contraction and biliopancreatic reflux.

In the future, it would be interesting to investigate the role of CCK antagonists in preventing post-ERCP pancreatitis and for comparison also to determine the levels of other peptide hormones in acute pancreatitis patients of different etiologies.

3 The role of microbes in acute pancreatitis

Microbes are cultured from the pancreas in 40-70 % of all patients suffering from necrotizing pancreatitis, and infection is the most common cause of mortality among these patients (Beger et al. 1986, Gerzof et al. 1987, Bassi et al. 1989). Although, experimental studies have shown that microbes may reach the pancreas via different routes (Tarpila et al. 1988, Widdison et al. 1990, Widdison et al. 1994), the route of infection in human acute pancreatitis is unclear. Furthermore, the microbiological mechanism of pancreatic injury in patients with post-ERCP pancreatitis has been previously suggested (Keynes 1987, Nordback and Airo 1988). However, in studies of post-ERCP pancreatitis little attention is paid to the possible effect of antibiotics on the development and the course of pancreatitis episode. Infectious complications, such as cholangitis and sepsis, in connection with ERCP have been widely studied, and antibiotic prophylaxis is recommended by many authors particularly for patients with severe jaundice or failure in decompression of the biliary tree (Niederau et al. 1994).

In the present study we investigated, whether the etiology of pancreatitis episodes had any quantitative or qualitative effect on the development of infection in pancreatic necrosis, and secondly whether antibiotic prophylaxis has any effect on post-ERCP pancreatitis.

First we investigated the microbiology of pancreatic necrosis in patients with necrotizing pancreatitis. Because of the retrospective nature of the study, the etiology of the pancreatitis episodes could be questioned. To increase the reliability of the assessment of the etiology of the pancreatitis episodes, we excluded patients with hyperlipidemia, acute cholecystitis and those with a subsequent diagnosis of pancreatic neoplasm, even when gallstones were present. Also patients with simultaneous gallstones and alcohol misuse were excluded. The diagnosis of necrotizing pancreatitis episodes can be considered reliable, because we only included operatively treated patients. To increase the reliability of the microbiological findings, we only included those patients with complete cultures taken at the first operation. Furthermore, we did not include patients from recent years, when fine needle biopsies were frequently conducted preoperatively, since the needle puncture may be a source of contamination. We found that pancreatic necrosis was more frequently infected in the gallstone pancreatitis patients compared to the alcoholic pancreatitis patients, although the extent of necrosis was not different. The bacterial spectrum in our study was similar to many previous studies (Bittner et al. 1987, Teerenhovi et al. 1988, Bassi et al. 1989, Büchler et al. 1992, Widdison and Karanjia 1993, Sainio et al. 1995, Friess et al. 1996), but when the etiology of pancreatitis episode was taken into account, we detected some qualitative differences between

gallstone and alcoholic pancreatitis. During the first week, pancreatic necrosis was more frequently contaminated with Gram-negative bacteria in gallstone pancreatitis than in alcoholic pancreatitis, whereas later in the course, Gram-positive bacteria, especially Staphylococci, were the most common bacteria cultured from the blood or necrosis in alcoholic pancreatitis. Sainio and colleagues suggested that catheters are the main route of contamination and sepsis in alcoholic pancreatitis (Sainio et al. 1995).

The reason for these differences in the microbes that contaminate pancreatic necrosis in gallstone and alcoholic pancreatitis is unknown. However, the bacterial spectrum in the necrosis of gallstone pancreatitis patients resembles that found in bile, perhaps even more than that found in feces. Furthermore, anaerobic bacteria, which predominate in the colon (Clostridium perfringens, Bacteroides fragilis) have only been isolated from 12-13 % of infected pancreatic necrosis in this as well as other studies (Friess et al. 1996). This data in common with the data shown in experimental studies (Widdison et al. 1994), suggest that pancreatic necrosis might also be infected via bile reflux in at least some patients suffering from acute gallstone pancreatitis.

Post-ERCP pancreatitis has usually been associated with manipulation-induced irritation. High injection pressure and precutting sphincterotomy are well known procedure-related factors, which increase the risk of post-ERCP pancreatitis (Freeman et al. 1996). In spite of efforts to avoid these risk factors, 5-7 % of patients undergoing ERCP still develop post-ERCP pancreatitis (Sherman and Lehman 1991, Sherman 1994, Freeman et al. 1996), which raises the question of other possible risk factors.

In only two randomized studies, investigating antibiotic prophylaxis in connection with ERCP, the incidence of post-ERCP pancreatitis is mentioned (Niederau et al. 1994, Hazel et al. 1996). In the study by Niederau and colleagues, they did not find a significant difference in the incidence of post-ERCP pancreatitis between the groups (3/50 vs 2/50, p=0.2). However, the patient population was relatively small and there were no data given regarding the definitions of post-ERCP pancreatitis diagnosis. In another randomized study by Hazel and colleagues, the incidence of acute post-ERCP pancreatitis was only 1.8 %, which is lower than the incidence in the other randomized studies. Obviously, the focus of either of these studies was not the prevalence of post-ERCP pancreatitis.

In this study, the patient population was different from the studies by Niederau and Hazel, since we excluded those patients with clinical jaundice or failed decompression.

We found an increased frequency of both pancreatitis and cholangitis in patients, who did not receive antibiotic prophylaxis before the ERCP. The incidence of post-ERCP pancreatitis was 6 %, reflecting what has been found in other randomized studies (Sherman and Lehman 1991, Sherman

1994, Freeman et al. 1996, Cavallini et al. 1996). We know that 60-70 % of patients undergoing ERCP have slightly elevated serum amylase activity, usually up to two fold, during the first 24 hours after ERCP, while only some of them develop clinical acute pancreatitis (Sherman and Lehman 1991, Sherman 1994, Chen et al. 1994, Cavallini et al. 1996). Our diagnosis of pancreatitis was based on serum amylase activity by three fold over the upper normal range, which is considered to have over 90 % specificity to detect pancreatitis (Lin et al. 1989), and on an increased CRP level, leukocytosis and the clinical picture. Early high or septic fever or an increase in liver function values were not allowed in the diagnosis of acute post-ERCP pancreatitis. Whilst the present study was not double-blind or placebo-controlled, we feel that the study plan was relevant, because the endoscopist was not aware of the group, the diagnosis of pancreatitis and cholangitis was based on laboratory test results and not the clinical picture alone. Furthermore, the data analysis was made by an independent investigator not participating in the patients treatment and the study groups appeared to be well matched supporting the successful randomization. Interestingly, all the patients in the control group with remarkably elevated serum amylase activity (> 900 IU/l), also developed clinical acute pancreatitis compared to only 44 % of the patients in the prophylaxis group. This may indicate that antibiotic prophylaxis prevents the development of pancreatic irritation into clinical acute pancreatitis in some patients.

The results of the present study suggest that antibiotic prophylaxis before ERCP diminishes the development of both pancreatitis and cholangitis, and can, thus, be routinely recommended. The results also suggest that bacteria may play an important role in the development of pancreatic irritation into clinical acute post-ERCP pancreatitis.

SUMMARY, CONCLUSIONS AND HYPOTHESIS

In the present study the role of the gallbladder, cholecystokinin and microbes were studied in human acute gallstone and post-ERCP pancreatitis.

The major findings and conclusions were:

1 The gallstone pancreatitis patients who still had the gallbladder in situ at the time of initiation of pancreatitis developed a severe episode of pancreatitis (according to Glasgow criteria) and complications more often than the gallstone pancreatitis patients who had previously undergone cholecystectomy.

The gallbladder, thus, may have an effect on the severity of acute gallstone pancreatitis, whereas in post-ERCP pancreatitis it is not important.

2 The circulating plasma CCK levels are elevated during the first hours after the ERCP in post-ERCP pancreatitis patients, and then gradually decrease over 24 hours to almost unmeasurable levels. In gallstone and alcoholic pancreatitis the CCK levels are similarly low after 24 hours from the beginning of symptoms and remain low until the patient is recovering well.

Thus, high serum CCK levels are associated with acute pancreatitis at least when induced by ERCP and low serum CCK levels are associated with the acute pancreatitis episode until recovery, independent on the etiology.

These findings suggest that CCK may have a role in the early, but not late in the course of acute pancreatitis, although secondary increase can not be ruled out.

3 The pancreatic necrosis was contaminated with Gram-negative bacteria during the first week more often in the gallstone pancreatitis patients than in the alcoholic pancreatitis patients. Thus, the bacterial spectrum contaminating necrosis is associated with the etiology of necrotizing pancreatitis.
This finding suggests that the route of microbes into the pancreas may be different in gallstone and alcoholic pancreatitis.

4 In a randomized prospective controlled study cephtazidime significantly reduced the development of post-ERCP pancreatitis. Cephtazidime also reduced the development of pancreatic irritation to the acute post-ERCP pancreatitis.
Routine antibiotic prophylaxis is recommended to patients scheduled for ERCP. These findings might suggest a microbial mechanism of pancreatic injury during ERCP.

Hypothesis

It is interesting to find that all the findings support, although not prove, the hypothesis that in gallstone pancreatitis pancreatic reflux by contaminated bile may affect the severity of the disease.

In post-ERCP pancreatitis pancreatic contamination by bacteria may be induced by cannulation and not by biliopancreatic reflux.
Thus, local contamination may be an important early event in gallstone and post-ERCP pancreatitis.

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ORIGINAL COMMUNICATIONS