ACUTE NECROTIZING PANCREATITIS IN RATS

Acute necrotizerende pancreatitis in ratten

PROEFSCHRIFT

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PROMOTOREN : PROF. J.H.P. WILSON
PROF. DR. J. JEEKEL

OVERIGE LEDEN: PROF. DR. I.L. BONTA
PROF. DR. J.L. TERPSTRA

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Wat heb ik dat ik niet gekregen heb?

aan mijn ouders

aan Hanneke

CONTENTS

ABBREVIATIONS USED:

AP	= acute pancreatitis
ANP	= acute necrotizing pancreatitis
PLA2	= phospholipase A2, an enzyme that causes the liberation of arachidonic acid, the precursor of prostaglandins, thromboxanes and leukotrienes
PGE2	= prostaglandin E2
PGI2	= prostaglandin I2 = prostacyclin
6-keto-PGF1alpha	= 6-keto-prostaglandin F1alpha, a bioconversion product of PGI2
TXA2	= thromboxane A2
TXB2	= thromboxane B2, a stable end product of TXA2
STC	= sodium taurocholate, a bile salt
SRIF	= somatostatin
SMS	= SMS 201-995 = a long acting somatostatin analogue

CHAPTER 1

INTRODUCTION AND AIM OF THE STUDY.

Acute pancreatitis can be divided clinically and morphologically into two forms: edematous pancreatitis and hemorrhagic necrotizing pancreatitis. The incidence of the necrotizing type is relatively low, but is reported to be on the increase (1). Acute necrotizing pancreatitis is associated with a high mortality rate (2). Early mortality occurs usually within one week of onset of the disease and is caused by systemic manifestations, such as shock, sepsis, respiratory insufficiency, renal insufficiency and hepatic failure; late mortality occurs three to five weeks after onset and is due to local complications such as abscesses and hemorrhage (3).

The pathogenesis and pathophysiology of acute necrotizing pancreatitis are not yet clear, despite intensive investigation. The generally accepted view is that pancreatic enzymes are activated prematurely, leading to autodigestion of the gland (4). Activated pancreatic enzymes may also enter the systemic circulation with possibly detrimental effects on almost every organ in the body. The pancreatic enzyme trypsin is thought to play a key role in the development of acute necrotizing pancreatitis, because it is able to activate all other pancreatic enzymes. However, the disappointing results of the trypsin inhibitor aprotinin (Trasylol (R)) as a modifier of both the local and systemic effects of acute pancreatitis have undermined the significance of trypsin (5). Much of the damage in acute necrotizing pancreatitis must be ascribed to other pancreatic enzymes such as elastase, lipase and phospholipase A2. Phospholipase A2, when prematurely activated, is able to cause coagulation necrosis of pancreatic cells. The action of phospholipase A2 on the phospholipids in cell membranes also leads to the liberation of arachidonic acid (6). Arachidonic acid is the major precursor of prostaglandins, thromboxanes and leukotrienes - the eicosanoids. Eicosanoids are biologically very active mediators, which are thought to play a role in the pathogenesis and pathophysiology of several diseases including endotoxic shock, pulmonary disorders, renal ischemia, sepsis and liver diseases (6). Eicosanoids exert a potent effect on cellular integrity as well as the microcirculation in several organs. As such they may mediate much of the damage to pancreatic cells seen in acute necrotizing pancreatitis.

Somatostatin, an inhibitor of pancreatic secretion, exerts a cytoprotective action in the splanchnic area (7). The action of somatostatin may be effectuated via the formation of certain eicosanoids.

Since eicosanoids occur in other organs through vascular dissemination as well as formation by circulating phospholipase A2 in non-injured tissues (8), they may also lead to symptoms of respiratory insufficiency, renal insufficiency and shock.

The specific aim of the present study was to investigate whether eicosanoids play a role in acute necrotizing pancreatitis. Because of the limited number of patients with acute pancreatitis admitted to the hospital each year, as well as the practical difficulties encountered in studying these patients, we had to turn to an experimental model of acute necrotizing pancreatitis. Experimental pancreatitis has been known since 1856 (9) when Claude Bernard injected a mixture of bile and olive oil into the pancreatic duct of a dog. We used a model based on the introduction of a bile salt into the pancreatic ducts of rats.

The purpose of the studies described in this thesis was:

1) to describe a model of experimental necrotizing pancreatitis in rats;
2) to determine whether eicosanoids are formed in acute necrotizing pancreatitis in rats;
3) to investigate whether inhibition of eicosanoid synthesis is important for the survival of rats with acute necrotizing pancreatitis;
4) to discover whether the specific balance between eicosanoids is important for the survival of rats with acute necrotizing pancreatitis; and
5) to determine whether a long-acting somatostatin analogue (SMS 201-995) has a beneficial effect in acute necrotizing pancreatitis in rats and, if so, whether such an effect can be related to changes in eicosanoid levels.

1.1. REFERENCES

1. Corfield AP, Cooper MJ, Williamson RCN. Acute pancreatitis: a lethal disease of increasing incidence. Gut 1985;26:724-729.

2. Eggink WF. De operatieve behandeling van pancreatitis. Thesis. Erasmus University. Rotterdam, 1981.

3. Ammann R. Acute pancreatitis. in: Gastroenterology, 3rd edition, edited by H.L. Bockus. W.B. Saunders Company, London, Volume 3, 1976, pp. 1020-1039.

4. Steer ML. Etiology and pathophysiology of acute pancreatitis. In: The exocrine pancreas: biology, pathobiology, and diseases, edited by V.L.W. Go, et al. Raven Press, New York, 1986, pp. 465-474.

5. Millat B, Zazzo J-F, Poynard T. Les essais therapeutiques randomises dans le traitement de la pancreatite aigue. Gastroenterol Clin Biol 1986;10:341-354

6. Ouwendijk RJT. Eicosanoids, endotoxins and liver disease. Thesis. Erasmus University. Rotterdam, 1985.

7. Szabo S, Usadel KH. Cytoprotection-organoprotection by somatostatin; gastric and hepatic lesions. Experientia 1982;38:254-256.

8. Flynn JT, Henry JM, Perkowski S. Phospholipase A2 stimulated release of prostanoids from the isolated perfused rabbit liver: implications in regional cellular injury. Can J Physiol Pharmacol 1981;59:1268-1273.

9. Davis conference: Geokas MC, moderator. Acute pancreatitis. Ann Intern Med 1985;103:86-100.

CHAPTER 2

ETIOLOGICAL FACTORS IN ACUTE PANCREATITIS.

2.1 INTRODUCTION

Acute pancreatitis in humans is a disease of variable intensity, ranging from mild edema to partial or generalized pancreatic necrosis. The acute form of pancreatitis has to be distinguished from the chronic variety. Acute and chronic pancreatitis are defined on the basis of clinical, functional and morphological criteria. Acute pancreatitis, an acute inflammatory condition typically presenting as abdominal pain, is usually associated with elevated levels of pancreatic enzymes in blood and urine. Chronic pancreatitis is a progressive inflammatory disease, characterized by irreversible morphological changes and associated with pain and/or permanent impairment of function (1,2).

Two main morphological forms of acute pancreatitis are distinguished: the edematous form and the necrotizing form. Edema may cease spontaneously but can also progress to necrosis for reasons not well understood (3). The events thought to lead to pancreatic necrosis are shown schematically in figure 1.

FIGURE 1: PROPOSED STEPS LEADING TO NECROSIS IN ACUTE PANCREATITIS.

Etiological factor (biliary tract disease, alcohol)

•

Initiating process (bile reflux, duodenal reflux,
complement activation, ischemia)

•

Initial pancreatic disorder (edema, vascular damage, loss
of duct integrity, acinar damage)

•

Activation of digestive enzymes (trypsin...phospholipase, elastase,
chymotrypsin, kallikrein; lipase)

•

Pancreatic necrosis

At present we have little information on the early changes in the course of the disease, and what we do know is based on experimental evidence. When the inflammation is severe, marked tissue necrosis of the gland develops and the pancreatic exudate, containing active enzymes and toxins, causes fat necrosis throughout the abdominal cavity. Fat necrosis can be found at the root of the mesentery, in the lesser peritoneal sac, retroperitoneum and kidney bed, as necrotic pathways on both sides of the aorta, and extending along the bed of the ureters as far as the true pelvis (4).

The clinical presentation of acute pancreatitis is governed to a large extent by the severity of the inflammatory process. At the onset of the disease distinction between the edematous and necrotizing variety may be impossible. In addition, a number of other intraabdominal illnesses need to be included in the differential diagnosis (5).

The complications of acute pancreatitis contribute to the mortality. In a study by Trapnell (3) 45 out of 76 patients died within a week of the onset of symptoms at which time the changes in the pancreas consisted mainly of edema and nonconfluent hemorrhage. The harmful consequences of chemical burn with large fluid and protein losses as well as the effects of circulating toxins and enzymes contribute to end-organ failure, which leads to death. For 17 out of 76 patients who died later in the course of the disease (after 3-5 weeks) problems in the pancreatic region itself had to be considered the major factors responsible for death.

2.2 ETIOLOGICAL FACTORS

Etiological factors known to produce acute pancreatitis in humans include biliary tract disease, ethyl alcohol abuse, a variety of other conditions and idiopathic causes. Biliary tract disease and alcoholism are described as the leading etiological culprits, whereas the other factors are relatively rare. Since a number of the rare factors usually occur in association with biliary tract disease and alcoholism they cannot always be considered a separate cause of acute pancreatitis.

The factors are summarized in table 1 (modified according to Warshaw AL, Richter JM (6)).

TABLE 1: POSSIBLE CAUSES OF ACUTE PANCREATITIS.

Mechanical block at ampulla:	Infectious causes:
a. gallstones	a. viruses
b. ampullary stenosis, sphincter disorders, pancreas divisum	b. bacteria c. parasites d. miscellaneous
c. duodenal disorders	
Vascular factors/ischemia:	Other causes:
a. circulatory shock	a. surgery
b. vasculitis	b. trauma
c. emboli	c. hereditary
d. malignant hypertension	d. sympathetic nerve stimulation
e. hypothermia	e. eating disorders
Toxic and metabolic factors:	f. pregnancy
	g. Scorpion venom
a. alcohol	h. allergic/ immunological factors
b. hypercalcemia	
c. hyperlipemia	
d. other metabolic causes	i. sarcoidosis
e. drugs	j. idiopathic

2.2.1 MECHANICAL BLOCK AT AMPULLA

(a) Biliary tract disease

There is a significant relationship between biliary tract disease and acute pancreatitis. About two-thirds of the patients with acute pancreatitis have gallstones (7,8), but fewer than 5% of the patients with gallstones develop acute pancreatitis. The disease is seen more frequently in females and markedly obese individuals. Small gallbladder stones, enlarged cystic ducts, impacted stones of a certain size and a functioning common channel are

predisposing factors in the development of gallstone pancreatitis (9). Patients with gallstone pancreatitis were found to have smaller and more numerous gallbladder stones, a wider pancreatic duct and a larger functioning common channel than those without gallstone pancreatitis (10). These differences in the biliary tree might facilitate the migration of calculi mechanically.

A study of the functional activity of Oddi's sphincter in patients with gallstone pancreatitis revealed manometric features of hypotonia (11). An incompetent Oddi's sphincter may enhance duodenal reflux.

a-1. Bile reflux

Bile reflux is thought to be the most important pathogenetic link between biliary and pancreatic disease. Bile reflux may occur when the ampulla is blocked and when there is a functioning common channel. The common channel theory was first proposed by Opie in 1901 (10).

However, impacted stones could not be found in the ampulla of Vater in the majority of cases and this hypothesis was more or less discarded. Interest in the bile reflux theory has been renewed by the work of Acosta and Kelly (8, 12-14). Acosta found impacted stones in the ampulla in 75% of his patients during surgery soon after the onset of acute pancreatitis (14). In addition it has been suggested that stones may pass on after transiently blocking the ampulla, causing edema and spasms. Bile microaggregates may irritate the papilla (10,15). Occult gallbladder microlithiasis causing acute pancreatitis has been described (16), as well as microlithiasis of the cystic duct (17). After an attack of acute pancreatitis, gallstones could be detected in the stools of 85-90% of the patients (12,13,18).

Surgical or endoscopic relief of an obstructed ampulla is followed by complete and immediate recovery of the pancreatitis (19-21). Recurrence of pancreatitis can be prevented by cholecystectomy (22).

A common channel is present in approximately 15% of normal subjects (10,23) and under normal conditions pancreatic duct reflux can occur without the development of acute pancreatitis. Reflux of contrast medium into the pancreatic duct during peroperative cholangiography has been observed in approximately 67% of the patients with gallstone pancreatitis (10,13). This was associated with a wider pancreatic duct forming a wider angle with the

common bile duct and with a larger functioning common channel (10,23,24). These differences in choledocho-pancreatic duct anatomy appear to facilitate the reflux of bile into the pancreatic duct mechanically.

Although bile reflux may be of pathogenetic importance, some questions remain. For example, acute pancreatitis can also occur when impacted stones block the common channel. In addition, the passage of normal bile through an unobstructed pancreatic duct system does not induce acute pancreatitis in experimental animals (25). Moreover, the pressure in the bile duct is lower than in the pancreatic duct and it does not seem very likely that the normal pressure relationship will change during brief obstruction of the papilla of Vater (15).

a-2. Pancreatic duct obstruction

It has also been suggested that common duct stones induce acute pancreatitis by obstructing the pancreatic duct. In a study of the opossum, obstruction of the common bile duct above the pancreatic duct caused hyperemia of the gland without pancreatitis. Obstruction below the pancreatic duct as well as simultaneous obstruction of both the common bile duct and the pancreatic duct induced severe acute necrotizing pancreatitis (26).

Continued pancreatic secretion into an obstructed pancreatic duct leads to ductal hypertension. Ductal hypertension has been suggested to be an important event in the development of acute pancreatitis in that it causes disruption of acinar and ductal integrity (27). However, simple occlusion of the pancreatic duct leads to edema and subsequently to atrophy, but not to actual pancreatitis (22); therefore additional factors must play a role.

(b) Obstruction at the level of the ampulla

Acute pancreatitis has been described in association with pancreatic duct calculi, tumors, metastasis (especially from bronchogenic carcinoma), inflammatory strictures, epithelial metaplasia, inflammation and edema of the papilla of Vater, motility disorders of Oddi's sphincter (29,30), juxta-ampullary diverticula and pancreas divisum (22,27,28).

Outflow obstruction in the presence of other pathogenetic factors (enhanced pancreatic secretion, vascular disorders, bile reflux) will facilitate the

development of pancreatitis (31).

b-1. Pancreas divisum

Pancreas divisum is defined as an embryologic failure of the fusion of the ventral and dorsal pancreatic ducts. The incidence of this anomaly is between 1 and 5% (22). A high frequency of recurrent pancreatitis among individuals with pancreas divisum has been reported in the literature (22,32); it is ascribed to the possibility that the ducts are too small to accept all pancreatic secretions. However the relationship between pancreas divisum and pancreatitis has been questioned (33,34) and perhaps pancreas divisum should be seen as a coincidental anatomical phenomenon.

(c) Duodenal disorders

Duodenal disorders described as etiological factors of acute pancreatitis include periampullary duodenal diverticula, periampullary polyps, penetrating duodenal/peptic ulcer, duodenal duplication, trichozoar, obstruction of the duodenum due to an annular pancreas, intraluminal diverticula, tumor growth, inflammatory stricture due, for example, to regional enteritis, arteriomesenteric occlusion and obstructed afferent loop after gastrectomy (22,27,28).

Duodenal reflux is suggested as the major pathogenetic mechanism in such cases, as it is in experimental acute pancreatitis in dogs induced by closing a duodenal loop (Pfeffer technique).

2.2.2. VASCULAR FACTORS AND ISCHEMIA

Although the pancreas has a very efficient blood supply, the organ, like the kidney, is highly vulnerable to ischemic necrosis. Shock and prolonged hypoperfusion of the pancreas can lead to the development of acute pancreatitis (35,36). In the experimental setting it is possible to induce acute pancreatitis by exposing the pancreas to severe ischemia (37).

Vascular disorders with reduced perfusion of the pancreas are also regular sequelae to severe acute pancreatitis, leading to further deterioration of the gland (22,38). Pancreatic circulation decreases rapidly in acute pancreatitis

(39-41). Ischemia due to hypoperfusion is a critical factor of the progression from edema to necrosis (35,41).

Ischemia may thus be an important determinate of the severity of an attack, also in pancreatitis associated with alcohol and biliary tract disease. Shock in acute pancreatitis is associated with activation of the coagulation system (42). Lysosomes, sensitive to a stimulus such as ischemia, become activated (43). Vasoactive mediators, including eicosanoids, may also be important (see Chapter 4).

Diffuse vascular disorders are associated with acute pancreatitis, apparently as a result of the ischemia (28). The disease states include necrotizing angiitis caused by amphetamines, periarteritis nodosa, systemic lupus erythematosus, malignant hypertension and thrombotic thrombocytopenic purpura (22,28,44). Ischemia may also play a role in the development of acute pancreatitis in patients with hypothermia and after open heart surgery (45-47)

2.2.3. TOXIC AND METABOLIC FACTORS.

a. Alcoholism

High alcohol intake over long periods (8-10 years) can lead to pancreatitis (22,27). It is said that over the past decades the frequency of acute pancreatitis due to alcoholism has increased, whereas that of acute pancreatitis due to biliary tract disease has decreased (22,48). However, though alcohol is of major importance in the etiology of chronic pancreatitis, its role in acute (reversible) disease has recently been challenged (1,2). The definition of acute alcoholic pancreatitis is a clinical one and depends on the reversibility to normal pancreatic morphology after an attack. The first manifestation of alcohol-induced chronic pancreatitis may be an episode of clinically acute pancreatitis, because exacerbations in the early phases closely resemble attacks of acute pancreatitis (1,2). True acute alcoholic pancreatitis is now thought to occur infrequently and the diagnosis must be reserved for those individuals who manifest evidence of an acute pancreatitis, after limited exposure to alcohol, for which no other cause can be found.

The mechanisms by which alcohol can induce acute pancreatitis are not fully understood. There is evidence that in chronic alcoholic pancreatitis certain

proteins in pancreatic juice precipitate, thereby forming a matrix for calcified pancreatic calculi (22). Protein plugs are believed to cause partial outflow obstruction. Alcohol also increases the tone of Oddi's sphincter (22). Oral consumption of alcohol causes a transient stimulation of pancreatic exocrine secretion (22,27,44). The combined effects of early stimulated secretion and outflow obstruction might explain alcohol-induced acute pancreatitis via the obstruction-hypersecretion theory.

It has also been suggested that bile reflux can take place more easily when there is decreased pancreatic secretion, which is a late effect of oral alcohol intake (27). In addition, alcohol may exert a direct toxic effect on the pancreas. An altered balance between proteolytic enzymes and protease inhibitors in pancreatic juice in vitro has been reported to be alcohol-related (27). Hyperlipemia, associated with alcohol, may also be of greater causal significance (49,50).

b. Hypercalcemia

Acute pancreatitis develops in 7% of the patients with hyperparathyroidism (22,27,28). One-quarter of these patients who undergo a hypercalcemic crisis develop acute pancreatitis, and hypercalcemia is thought to play a key role in the induction of acute pancreatitis. Acute pancreatitis is also associated with hypercalcemia of other origins, such as familial hypocalciuric hypercalcemia (28), total parenteral nutrition (22,28), hypervitaminosis D (51) and metastatic bone disease (51). Hypercalcemia may cause obstruction of ducts through the formation of stones. The frequency of such calcifications is considerably higher than in acute pancreatitis of other etiology since 25-45% of the patients develop parenchymal calcinosis or ductal stones (22,27). In addition, calcium may activate trypsin, stimulate enzyme secretion and induce a vasculitis within the pancreas (22).

Recently, the causal relationship between hyperparathyroidism and acute pancreatitis was challenged (52-55). The true incidence of the association was reported to be less than 1.5%. Cure of hyperparathyroidism was not always associated with resolution of the pancreatitis unless other etiological factors such as gallstones were corrected (53-55).

c. Hypertriglyceridemia

The familial hyperlipoproteinemias (Frederickson types I,IV and V) are associated with pancreatitis (44). Hyperlipemia is sometimes seen in alcoholics and may be an etiological factor of great importance. It also develops as a consequence of taking estrogen-containing contraceptive pills (51). Experimental pancreatitis in dogs was intensified when a high lipid diet was given beforehand (22). Possible mechanisms of action are the embolization of agglutinated serum lipid particles, acute fatty infiltration of the acinar cells and release of free fatty acids from serum triglycerides by the activity of lipase. Free fatty acids may induce the formation of local microthrombi that damage the capillary membrane, thereby producing ischemic injury (22,27). Free fatty acids may also have a direct toxic effect on the pancreatic parenchyma (22).

d. Other metabolic causes

Acute pancreatitis has been related to liver failure, chronic uremia and diabetic coma (22).

e. Drugs

Many drugs that are able to cause pancreatitis have been described and the list continues to grow. The mechanisms and reversibility of these effects remain uncertain in many cases. The following drugs definitely cause acute pancreatitis: azathioprine, thiazides, furosemide, sulfonamides, tetracycline, estrogens, valproic acid and L-asparaginase (56,57). The thiazides, furosemide, sulfonamides and L-asparaginase may have a direct toxic action on the pancreatic parenchyma, whereas azathioprine and the sulfonamides may induce an allergic immune response. Estrogens cause a hyperlipoproteinemia. Diuretics could alter the viscosity of the pancreatic juice, affect calcium metabolism or cause ischemia. Tetracycline leads to alterations in liver cells. Azathioprine may also activate latent infections as well as cause ischemia (56,57).

There is less convincing, but still suggestive, evidence for a causal relationship with 5 other drugs: corticosteroids, chlorthalidon, ethacrynic

acid, phenformin and iatrogenic hypercalcemia (56,57). Due to inadequate or contradictory evidence, the link between a number of additional drugs and acute pancreatitis is considered possible, conditional or doubtful.

Intoxication with antiacetylcholinesterase-containing insecticides also causes acute pancreatitis (27).

2.2.4 INFECTION

A number of viruses, bacteria and parasites have been reported to cause acute pancreatitis. The viruses responsible for mumps, infectious mononucleosis and hepatitis as well as cytomegalovirus, adenovirus and the Coxsackie B, Eppstein Barr and ECHO viruses have been associated with acute pancreatitis (22,27,28). Bacterial associations include Salmonella, Streptococcus (22,28), Campylobacter (58) and Yersinia (59). Acute pancreatitis has also been described following infections with Legionella (60,61), Chlamydia trachomatis (62) and Mycoplasma pneumoniae (22,27). The migration of Ascaris lumbricoides into the pancreatic duct, thereby causing acute pancreatitis, has been described (22,27,28). Clonorchis sinensis has been recovered from the pancreatic duct of a patient with acute pancreatitis (27).

Acute pancreatitis has been described following vaccinations with monovalent typhoid and cholera vaccines (63).

2.2.5 OTHER CAUSES

a. Surgery

Acute pancreatitis has been described after most kinds of surgery, particularly biliary, gastric and splenic operations (64) and diagnostic procedures such as endoscopic retrograde cholangiopancreatography. However, acute pancreatitis may also result from surgery in areas far removed from the pancreas, including hysterectomy, neurosurgical procedures (27,64), cardiopulmonary bypass (45,46) and translumbar aortography (27,51). Many mechanisms have been considered, including local trauma of the pancreas, an obstructing long distal leg of a T-tube after choledochal exploration, low cardiac output status with hypoperfusion and ischemia of the pancreas and

interference with the flow of pancreatic juice (27,64).

a-1. Renal transplantation

Acute pancreatitis may develop after renal transplantation. The incidence is reported to be 2-7% (22,27). The acute pancreatitis, associated with a high mortality, may be attributable to several mechanisms: administration of corticosteroids and immunosuppressive drugs, viral infections, vasculitis and hypercalcemia.

b. Trauma

Acute pancreatitis has been described after both blunt and penetrating trauma (28).

c. Hereditary

A number of families with recurrent acute pancreatitis have been described. It has been suggested that an increased tendency to form protein plugs is present in these patients (22,27).

d. Sympathetic nerve stimulation

The autonomic nervous system influences pancreatic secretion, the outflow of pancreatic juice and the pancreatic blood supply. Experimentally, moderate pancreatitis may change into acute necrotizing pancreatitis after repeated vigorous stimulation of the sympathetic nerves (22). Mallet and Guy produced acute pancreatitis by stimulating the left splanchnic nerve (15). There is, however, no proof that a true neurogenic pancreatitis exists.

e. Eating disorders

Anorexia and bulimia may lead to acute pancreatitis (28). Acute pancreatitis is seen in individuals who overeat after prolonged fasting (51). The variation in pancreatic secretory output may play a role in its induction.

f. Pregnancy

Pregnancy used to be considered a risk factor for the development of acute pancreatitis. Since a number of the causes of acute pancreatitis usually occur during pregnancy, i.e. gallstones, hyperlipemia, administration of thiazides and increased stasis in duodenum and biliary tract, it is recognized that pregnancy itself should no longer be considered a separate cause of acute pancreatitis (27).

g. Scorpion venom

The sting of Tityus trinitatis leads to acute pancreatitis (22). Experimentally, administration of scorpion venom to dogs causes acute pancreatitis. The mechanism of action is thought to be stimulation of exocrine pancreatic secretion by a toxin in scorpion venom with a simultaneous relative obstruction of outflow (27).

h. Allergic and immunological factors

After induction of a local Sanarelli-Schwartzmann phenomenon extensive damage of the smaller pancreatic vessels developed (22). Acute pancreatitis is also associated with autoimmune factors. Acinar cell antibodies were demonstrated by direct immunofluorescence, but this was presumed to be an epiphenomenon rather than an immunological basis for pancreatitis (27).

i. Sarcoidosis

Pancreatitis due to the presence of granulomatous infiltration of the pancreas in sarcoidosis has been described (65).

j. Idiopathic causes

The etiology of acute pancreatitis remains obscure in a number of cases. However, with improved diagnostic procedures the causes may yet become clear in many cases of unknown etiology (20,29,66). At present, with careful study, only 5-7% of the patients with acute pancreatitis have idiopathic disease (27).

2.3. REFERENCES

1. Sarner M. Pancreatitis: definitions and classification. in: The exocrine pancreas, biology, pathobiology, and diseases, edited by V.L.W. Go, et al. Raven Press, New York, 1986, pp. 459-464.

2. Sarles H. Revised classification of pancreatitis - Marseille 1984. Dig Dis Sci 1985;30:573-574.

3. Ammann R. Acute pancreatitis. in: Gastroenterology, 3rd edition, edited by H.L. Bockus. W.B. Saunders Company, London, Volume 3, 1976, pp. 1020-1039.

4. Love L, Meyers MA, Churchill RJ, et al. Computed tomography of extraperitoneal spaces. Am J Roentgenol 1981;136:781-789.

5. Banks PA. Acute pancreatitis: clinical presentation. in: The exocrine pancreas, biology, pathobiology, and diseases, edited by V.L.W. Go, et al. Raven Press, New York, 1986, pp. 475-479.

6. Warshaw AL, Richter JM. A practical guide to pancreatitis. Curr Probl Surg 1984;21:7-15.

7. Becker V. Pathological anatomy and pathogenesis of acute pancreatitis. World J Surg 1981;5:303-313.

8. Kelly TR. Gallstone pancreatitis. Arch Surg 1974;109:294-297.

9. Kelly TR. Gallstone pancreatitis. Ann Surg 1984;200:479-485.

10. Armstrong CP, Taylor TV, Jeacock J, Lucas S. The biliary tract in patients with acute gallstone pancreatitis. Br J Surg 1985;72:551-555.

11. Cuschieri A, Cumming JGR, Wood RAB, Baker PR. Evidence for sphincter dysfunction in patients with gallstone associated pancreatitis: effect of ceruletide in patients undergoing cholecystectomy for gallbladder disease and gallstone associated pancreatitis. Br J Surg 1984;71:885-888.

12. Acosta JM, Ledesma CL. Gallstone migration as a cause of acute pancreatitis. N Engl J Med 1974;290:484-487.

13. Kelly TR. Gallstone pancreatitis: pathophysiology. Surgery 1976;80:488-492.

14. Acosta JM, Pellegrini CA, Skinner DB. Etiology and pathogenesis of acute biliary pancreatitis. Surgery 1980;88:118-125.

15. Lange JF. Experimental studies on bilesalt-induced acute pancreatitis in the rat. Thesis. University of Amsterdam. Amsterdam, 1987.

16. Negro P, Flati G, Flati D, et al. Occult gallbladder microlithiasis causing acute recurrent pancreatitis. Acta Chir Scand 1984;150:503-506.

17. Kaufman Z, Spitz B, Dinbar A. Microlithiasis of the cystic duct. Am J Gastroenterol 1986;81:303-304.

18. Acosta JM, Rossi R, Ledesma CL. The usefulness of stool screening for diagnosing cholelithiasis in acute pancreatitis. A description of the technique. Am J Dig Dis 1977;22:168-172.

19. Safrany L, Cotton PB. A preliminary report: urgent duodenoscopic sphincterotomy for acute gallstone pancreatitis. Surgery 1981;89:424-428.

20. Siegel JH, Tone P, Menikeim D. Gallstone pancreatitis: pathogenesis and clinical forms - the emerging role of endoscopic management. Am J Gastroenterol 1986;81:774-778.

21. Neoptolemos JP, London N, Slater ND, et al. A prospective study of ERCP and endoscopic sphincterotomy in the diagnosis and treatment of gallstone associated pancreatitis. Arch Surg 1986;121:697-702.

22. Creutzfeldt W, Lankisch PG. Acute pancreatitis: etiology and pathogenesis. in: Bockus' Gastroenterology, 4th edition, J.E. Berk, editor-in-chief, W.B. Saunders Company, London, 1985, pp. 3971-3992.

23. Police AM, Waxman K, Smolin M, et al. Development of gallstone pancreatitis. The role of the common channel. Arch Surg 1984;119:1299-1300.

24. Armstrong CP, Taylor TV. Pancreatic-duct reflux and acute gallstone pancreatitis. Ann Surg 1986;204:59-64.
25. Robinson TM, Dunphy JE. Continuous perfusion of bile protease activators through the pancreas. JAMA 1963;183:530-533.
26. Senninger N, Moody FG, Coelho JCU, Van Buren DH. The role of biliary obstruction in the pathogenesis of acute pancreatitis in the opossum. Surgery 1986;99:688-693.
27. Steer ML. Etiology and pathophysiology of acute pancreatitis. in: The exocrine pancreas, biology, pathobiology, and diseases, edited by V.L.W. Go, et al. Raven Press, New York, 1986, pp. 465-474.
28. Fayne SD, Barkin JS. Acute pancreatitis: update 1986. Mt Sinai J Med 1986;53:396-403.
29. Toouli J, Roberts-Thomson IC, Dent J, Lee J. Sphincter of Oddi motility disorders in patients with idiopathic recurrent pancreatitis. Br J Surg 1985;72:859-863.
30. Guelrud M, Siegel JH. Hypertensive pancreatic duct sphincter as a cause of pancreatitis. Dig Dis Sci 1984;29:225-231.
31. McCutcheon AD. A fresh approach to the pathogenesis of pancreatitis. Gut 1968;9:296-310.
32. Warshaw AL, Richter JM, Shapiro RH. The cause and treatment of pancreatitis associated with pancreas divisum. Ann Surg 1983;198:443-452.
33. Delhaye M, Engelholm L, Cremer M. Pancreas divisum: congenital anatomic variant or anomaly? Contribution of endoscopic retrograde dorsal pancreatography. Gastroenterology 1985;89:951-958.
34. Ott H, Rosch W. Pancreas divisum - ursache einer pankreatitis? Med Welt 1983;34:2-4.
35. Warshaw AL, O'Hara PJ. Susceptibility of the pancreas to ischemic injury in shock. Ann Surg 1978;188:197-201.
36. Dreiling DA, Koller M, Su C-H. Vascular pancreatitis: a neglected disorder. Mt Sinai J Med 1986;53:482-484.
37. Broe PJ, Zuidema GD, Cameron JL. The role of ischemia in acute pancreatitis: studies with an isolated perfused canine pancreas. Surgery 1982;91:377-382.
38. Goodhead B. Acute pancreatitis and pancreatic blood flow. Surg Gyn Obstet 1969;69:331-340.
39. Becker H, Vinten-Johansen J, Buckberg GD, Bugyi HI. Correlation of pancreatic blood flow and high-energy phosphates during experimental pancreatitis. Eur Surg Res 1982;14:203-210.
40. Papp M, Makara GB, Hajtman B, Csaki L. A quantitative study of pancreatic blood flow in experimental pancreatitis. Gastroenterology 1966;51:524-528.
41. Donaldson LA, Williams RW, Schenk WG. Experimental pancreatitis: effect of plasma and dextran on pancreatic blood flow. Surgery 1978;84:313-321.
42. Becker V, Stollhoff K. Shock and terminal pancreatitis. Path Res Pract 1985;179:512-516.
43. Lefer AM. Minireview: The role of lysosomes in circulatory shock. Life Sci 1976;19:1803-1810.
44. Davis conference: Geokas MC, moderator. Acute pancreatitis. Ann Intern Med 1985;103:86-100.
45. Haas GS, Warshaw AL, Daggett WM, Aretz HT. Acute pancreatitis after cardiopulmonary bypass. Am J Surg 1985;149:508-514.
46. Aziz S, Bergdahl L, Baldwin JC, et al. Pancreatitis after cardiac and cardiopulmonary transplantation. Surgery 1985;97:653-660.
47. Rose DM, Ranson JHC, Cunningham JN, Spencer FC. Patterns of severe pancreatic injury following cardiopulmonary bypass. Ann Surg 1984;199:168-172.

48. Svensson JO, Nordback B, Bockey EL, Edlund Y. Changing pattern in aetiology of pancreatitis in an urban Swedish area. Br J Surg 1979;66:159-161.
49. Cameron JL, Zuidema GD, Margolis S. A pathogenesis for alcoholic pancreatitis. Surgery 1975;77:754-763.
50. Dickson AP, Imrie CW. Hyperlipidaemia, alcohol abuse and acute pancreatitis. Br J Surg 1984;71:685-688.
51. Wormsley KG. Diseases of the pancreas. in: Oxford textbook of medicine, 2nd edition, edited by Weatherall DJ, Ledingham JGG, Warrell DA, Oxford, 1987, Section 12, pp. 183-188.
52. Van Lanschot JJB, Bruining HA. Primary hyperparathyroidism and pancreatitis. Neth J Surg 1984;36:38-41.
53. Bess MA, Edis AJ, Van Heerden JA. Hyperparathyroidism and pancreatitis. Chance or causal association? JAMA 1980;243:246-247.
54. Watson RGK, Van Heerden JA, Grant CS, Klee GG. Postoperative hyperamylasemia, pancreatitis and primary hyperparathyroidism. Surgery 1984;96:1151-1157.
55. Shearer MG, Imrie CW. Parathyroid hormone levels, hyperparathyroidism and acute pancreatitis. Br J Surg 1986;73:282-284.
56. Mallory A, Kern F. Drug-induced pancreatitis: a critical review. Gastroenterology 1980;78:813-820.
57. Dobrilla G, Felder M, Chilovi F. Medikamentos induzierte akute pankreatitis. Schweiz Med Wschr 1985;115:850-858.
58. Bar BMAM, Van Dam FE. Een patient met een door Campylobacter veroorzaakte pancreatitis. Ned Tijdschr Geneesk 1985;129:2123-2135.
59. Lindholt J, Hansen PT. Yersiniosis as a possible cause of acute pancreatitis. Acta Chir Scand 1985;151:703.
60. Michel O, Naeije N, Csoma M, Sergysels R, De Coster A. Legionnaire's disease induced an acute pancreatitis. Eur J Respir Dis 1985;66:62-64.
61. Bollaert PE, Maurizi M, Laprevote-Heuly MC, Lambert H, Larcan A. Pancreatite aigue necrotico-hemorrhagique au cours d'une maladie des legionnaires. Presse Med 1986;15:1732.
62. Baccino E, Le Guillou M, Mottier D, et al. Pancreatite aigue et infection a Chlamydia trachomatis. Gastroenterol Clin Biol 1985;9:951.
63. Gatt DT. Pancreatitis following monovalent typhoid and cholera vaccinations. Br J Clin Pract 1986;40:300-301.
64. Martins Job J. Pancreatic injuries, postoperative pancreatitis and pancreatic fistulas. in: Gastroenterology, 3rd edition, edited by H.L. Bockus. W.B. Saunders Company, London, Volume 3, 1976, pp. 1081-1101.
65. McCormick PA, Malone D, Fitzgerald MX, Fitzgerald O. Pancreatitis in sarcoidosis. Br Med J 1985;290:1472-1473.
66. Feller ER. Endoscopic retrograde cholangiopancreatography in the diagnosis of unexplained pancreatitis. Arch Intern Med 1984;144:1797-1799.

CHAPTER 3

PATHOGENESIS OF ACUTE PANCREATITIS.

3.1. INTRODUCTION

Etiological factors may affect the pancreatic cells by different mechanisms, although many of the latter are speculative. For example, bile reflux, reflux of duodenal contents, ischemia and obstruction with overstimulation of enzyme secretion causing ductal hypertension have been suggested (1-5). In addition, Seelig (6,7) demonstrated the deposition of the C3 cytolytic component of complement in the pancreas during experimental acute pancreatitis and suggested that this may be the mechanism by which pancreatic cells are damaged. It is also possible that spontaneous intrapancreatic activation of digestive enzymes is responsible for the destruction of pancreatic cells (8-10).

In the initial phase of the disease, the integrity of vessels, acini and/or the duct system is affected. It is generally presumed that transformation of the initial condition into acute necrotizing pancreatitis arises from activation of the digestive enzymes, which causes autodigestion of the gland. This was originally suggested by Chiari (4). Experimental studies have shown that at least one of the pancreatic enzymes (phospholipase A2) will induce necrotizing pancreatitis after ductal or intraparenchymal injection, especially when combined with bile or bile acids (2).

A variety of factors such as endotoxins, exotoxins, viral infections, ischemia, anoxia, direct trauma, thrombin, cathepsin B, enterokinase, complement, bacterial enzymes and autoactivation are believed to be able to activate the digestive enzymes, depending on the strength and duration of the stimulus.

The escape of activated enzymes into the pancreatic parenchyma can cause local damage by direct destruction of the tissues. This leads to further edema, ischemia and inflammatory changes. Exudation into surrounding tissues as well as the peritoneal cavity creates a fluid rich in activated enzymes, histamine-releasing factors and vasoactive amines (2,11). When absorbed or released into the systemic circulation, a cascade of proteolytic enzyme systems in the body will be activated resulting in damage to other organs (12).

3.2. MECHANISMS OF THE INITIAL PANCREATIC DISORDER

3.2.1. Ischemia

It is presumed that changes in the pancreatic microcirculation lead to ischemia, which in turn can result in the development of the initial pancreatic disorder as well as the progression of mild pancreatitis to severe pancreatitis with necrosis (8,11,13).

Ischemia may result from a reduction in regional blood flow (11) or local disturbance of flow within the pancreas (14). There is experimental evidence that impairment of pancreatic blood flow leads to acute pancreatitis. Smyth (1940) demonstrated the development of pancreatic necrosis after injection of mercury droplets into the pancreatic arteries (15). It is possible to induce acute pancreatitis by exposing the pancreas to periods of severe ischemia (16) or injecting microspheres into the pancreatic arteries (Pfeffer, 1962) as well as by arterial occlusion (Popper, 1948) or venous ligation (Adams, 1953) (13). Experimentally induced hypotension is associated with a marked reduction in pancreatic blood flow, which in turn is associated with injury to the acinar cells (17). Lysosomes are sensitive to ischemia and become activated (18). In experimental acute edematous pancreatitis few vascular changes are seen, whereas in acute necrotizing pancreatitis poor capillary filling is noted with local extravasation of erythrocytes (19,20). Thal (1954) observed, by means of a transilluminating device, segmental spasms of the lobular arteries and veins with sluggish capillary flow (14). In addition Schiller reported that vasodilatation appeared to develop in the less affected areas (14). Vasodilatation suggests a redistribution of blood flow with hyperperfusion of minimally involved areas and stasis in the inflamed part of the pancreas. Endothelial disruption of capillaries or vascular thrombosis is thought to occur in acute pancreatitis (19-21). Plugging of the vessels by thrombin may result from intravascular coagulation (11).

Conversely, enhancement and protection of the pancreatic blood flow, especially the pancreatic microcirculation, repairs and prevents damage to the pancreas (11,22,23). Postganglionic sympathetic denervation (24) as well as the administration of low molecular weight dextrans (25,26) appears to have a favorable influence.

Superoxide radicals may mediate much of the damage caused by ischemia (27,28). The eicosanoid cascade can both activate and be activated by the generation of free radicals (29). Eicosanoids may thus play an important role in the pathogenesis of acute pancreatitis (see Chapter 4).

3.2.2. Reflux of duodenal contents

Reflux is normally prevented by a) a high secretion pressure in the pancreas and b) the pressure gradient between the pancreatic duct and the duodenum that is maintained by Oddi's sphincter. This natural defense mechanism may be destroyed by inflammation, functional disturbances of the sphincter muscle (30) and increased pressure in the duodenum due, for example, to a tumor (1,31). The duodenum contains a mixture of enterokinase, activated pancreatic enzymes, bile acids, lysolecithin, emulsified fat and bacteria, some of which are known to produce pancreatitis experimentally (4). When the pancreatic duct was ligated in the closed duodenal loop model of experimental acute pancreatitis, pancreatitis did not develop (4). In addition when a synthetic trypsin inhibitor was added to the duodenal contents pancreatitis did not develop (4). The reflux of duodenal contents may cause loss of pancreatic duct integrity. Bile salts in particular can play a role in this respect (32,33). Experimentally, the main pancreatic duct may become permeable following oral ingestion of aspirin and ethanol (34).

3.2.3. Reflux of bile

Reflux of bile may occur when there is obstruction at the level of the ampulla in combination with a functioning common channel. Reflux of bile as a pathogenetic event was suggested as long ago as 1901 by Opie. Bile, especially infected bile, and bile salts can alter the integrity of the ductal mucosal barrier, allowing the ductal contents to reach the parenchyma (33,35). Bile acids may damage pancreatic cells as a result of their detergent action. In infected bile deconjugation of bile acids occurs, producing substances with mucolytic and cytotoxic properties (4,35). Disturbances of the liver leading to abnormal bile production may also play a role in initiating pancreatic disease (36).

Bile acids may lead to spontaneous activation of minute amounts of

trypsinogen within the pancreas (37). An increase in the lysolecithin content (a consequence of the action of active phospholipase on bile lecithin) has also been demonstrated (37,38). Thus, bile acids are believed to increase the enzymatic activity of (at least) phospholipase A2 (39).

3.2.4. Activation of the complement system

Local antibody-dependent or independent activation of the complement system was followed by acute pancreatitis (6,7). The deposition of the C3 component of complement, which causes lysis of cell membranes, could be shown in experimental acute pancreatitis and in humans (6). Ductal injection of complement leads to acute pancreatitis with necrosis (6,7). Complement activation may thus be responsible for the initial pancreatic condition (damaged acinar cells, duct cells and/or vessel walls) although a causative role is not generally accepted (4,40).

3.2.5. Overstimulation of enzyme secretion

Ductal rupture may occur as a result of ductal hypertension. As a consequence extravasation of pancreatic juice into the parenchyma will occur and premature activation of enzymes may follow (8-10). Ductal hypertension may lead to ductal rupture but may also prevent the secretion of digestive enzymes by acinar cells (8). Experimental findings indicate severe changes in the specificity of the intracellular membrane fusion process after vigorous stimulation of pancreatic secretion (4). It has been suggested that the stimulation of enzyme output, not water and bicarbonate secretion, is important (4). However, the use of secretion-inhibiting agents, such as glucagon and calcitonin, did not improve the outcome in either experimental acute pancreatitis or prospective clinical trials (41-43).

Hypersecretion alone is unlikely to cause acute pancreatitis as it is difficult to induce pancreatitis by hypersecretion in experimental models (11), although in clinical practice some cases of acute pancreatitis develop as a result of hypersecretion alone (see Chapter 2). Obstruction of the pancreatic duct alone is also unlikely to cause acute pancreatitis. Obstruction of the duct leads to edema and subsequent atrophy of the gland, but not acute pancreatitis (11). There is experimental evidence in rats and

dogs that obstruction of the pancreatic duct in combination with stimulation of pancreatic exocrine secretion results in pancreatic necrosis, but only when bile reflux is possible (4).

3.3. INTRAPANCREATIC ACTIVATION OF DIGESTIVE ENZYMES

Activated forms of pancreatic enzymes have been detected in the pancreatic parenchyma, pancreatic juice and ascitic fluid of some patients with acute pancreatitis (4,5,8,44,45). An important issue that deserves attention is the mechanism responsible for the intrapancreatic activation of digestive enzymes.

3.3.1. Protective mechanisms

There are some intrinsic protective mechanisms that have to be overcome before activation of pancreatic enzymes can take place. These mechanisms are:

a. the harmful proteolytic and lipolytic enzymes are synthetized as inactive zymogens (trypsinogens, chymotrypsinogens, procarboxypeptidases, proelastases, prophospholipases), whereas less harmful digestive enzymes are synthetized in the active form (amylases, lipases, nucleases). Zymogens become activated by the enzymatic splitting of a peptide chain, but normally not before the duodenal lumen is reached (4).

b. the proenzymes are stored in zymogen granules that are isolated by a phospholipid membrane in the acinar cell (8-10).

c. protease inhibitors are present in pancreatic tissue, pancreatic juice and serum. The protease inhibitors normally prevent activation of zymogens and inactivate prematurely activated proteases until the enzymes reach the duodenal lumen (4,8,46).

d. the ductal epithelium is protected by a coating of mucopolysaccharides: a layer of mucus (4,47).

An abnormality of one or more of these factors may play an important role in the initiation and evolution of the disease. For example the antiprotease concentrations (especially alpha 1-antitrypsin and alpha 2-macroglobulin) may be insufficient. Active trypsin, normally bound to alpha 1-antitrypsin, is inactivated by this binding. Theoretically, low levels of the inhibitors may

occur under certain circumstances, such as shock, postoperative states and drug or alcohol ingestion, allowing premature activation of pancreatic enzymes.

The alpha 1-antitrypsin complex is associated with an alpha 2-macroglobulin, that is cleared by the reticuloendothelial system. Saturation of the reticuloendothelial system may lead to acute pancreatitis (11,48,49), whereas enhancement appears to confer protection (48). It has been shown that biliary obstruction impairs the functioning of the hepatic reticuloendothelial system (50).

The pancreatic duct normally functions as a barrier (32). Bile salts, aspirin and ethanol are able to increase the permeability of the duct (33,34). After bile salt perfusion of the pancreatic duct in concentrations present in duodenal contents, the duct becomes permeable to molecules as large as 20,000 daltons (33). Pancreatic enzymes such as phospholipase, trypsin and chymotrypsin are of about this size. Sterile human bile at low pressures caused moderate damage when injected into the rat biliopancreatic duct, whereas either sterile human bile at high pressure or infected bile at any pressure caused marked damage to the duct and led to almost complete loss of duct integrity (35). High pressure alone or bacterial solutions alone had little effect on duct integrity (35).

3.3.2. Activation of trypsin

Many authors propose a key role for trypsin in the pathogenesis of acute pancreatitis. Though at best only minute amounts of active trypsin can be detected in acute pancreatitis, they suggest that the autodigestive process is initiated by trypsin. Trypsin is able to activate all known pancreatic zymogens; only lipase (already secreted in its active form) is independent of trypsin. For a long time it was not possible to detect active trypsin in pancreatic tissue during acute pancreatitis, but with new immunochemical techniques complexes consisting of active trypsin and serum trypsin inhibitors could be demonstrated in human ascitic fluid, lymph and plasma (12,51).

Normally, trypsin is formed from trypsinogen in the duodenal lumen by the brush-border enzyme enterokinase. Enterokinase enters the duct system of the pancreas when duodenal reflux occurs. However, retrograde injection of enterokinase into rats did not cause either acute pancreatitis or activation of trypsinogen within the gland (4). Duodenal reflux after, for example,

sphincteroplasty or a pancreatico-jejunal anastomosis is usually not associated with acute pancreatitis. Thus activation of trypsin within the duct cannot be the sole prerequisite for the development of acute pancreatitis.

Some factors of the complement system can destroy the acinar cell membranes and activate trypsin (6,7). Thrombin is able to activate trypsin at a physiological pH (4). Lysosomal cathepsin B can activate trypsin, although the pH must be low (4,9,10). Ischemia may cause local acidosis and a low pH. Activation of trypsin by lysosomal hydrolases may occur within the acinar cell itself as a result of the admixture of digestive enzyme zymogens and lysosomal hydrolases, according to Steer and co-workers (9,10). The cytomembranes of the acinar cells in rats contain large amounts of trypsin inhibitor, whereas the zymogen granules do not (4).

3.3.3. Activation of chymotrypsin

Traces of chymotrypsin activity have been detected in human pancreatic juice during an attack of acute pancreatitis. Spontaneous activation of chymotrypsin in pure inactive juice may occur (4).

Chymotrypsin may activate the enzyme xanthine-oxidase, that can promote the formation of oxygen-free radicals (27,28). The eicosanoid cascade can both activate and be activated by the generation of free radicals (29). Experimental evidence from the isolated perfused pancreas model suggests that the generation of oxygen-derived free radicals may lead to the endothelial lesions that occur in acute pancreatitis (27). This destruction of capillary endothelium is thought to be an important step in the pathogenesis of acute pancreatitis. Scavengers of oxygen-free radicals decrease the severity of acute pancreatitis in an ex vivo model (52). A suggested source of the free radicals is the pancreatic cell or the vascular endothelium (53).

3.3.4. Activation of phospholipase

Phospholipase A activity has been demonstrated in pancreatic tissue, blood and ascitic fluid in experimental animals and humans with acute pancreatitis; moreover the level of activity was correlated with the severity of the attack (38,54,55). Activation of phospholipase is thought to occur mainly by trypsin (39). Spontaneous activation of phospholipase has, however, been demonstrated

in pure, inactive human pancreatic juice as well as isolated zymogen granules (37).

Creutzfeldt and Schmidt as well as Nevalainen have reviewed the role of phospholipase A as a pathogenetic factor in acute pancreatitis (38,39). Phospholipase A2 destroys cellular membranes by splitting membrane phospholipids into lysophospholipids; this may be an initiating event in acute pancreatitis. Bile acids are considered to be activators of phospholipase A (1,38), because they cause an increase in lysolecithin formation which must be due to the intrapancreatic activation of phospholipase A (37). Phospholipase A2 is also highly dependent upon calcium. The interaction between phospholipase A2 and membrane phospholipids leads to the formation of prostaglandins and leukotrienes, which may have an important effect on local homeostasis (see Chapter 4).

3.3.5. Activation of lipase

Lipase is excreted in its active form in pancreatic juice. Lipase can cause severe necrosis of the pancreatic parenchyma and is also held responsible for the fat necrosis that develops in acute pancreatitis (56,57). With immunocytochemical and ultrastructural methods peripancreatic fat necrosis was identified as one of the earliest visible lesions in human acute pancreatitis. Acinar cell necrosis and hemorrhage were regarded as secondary changes (58).

3.4. PANCREATIC ENZYMES AND THE AUTODIGESTIVE PROCESS

3.4.1. Introduction

In the edematous form of acute pancreatitis the pancreas is enlarged and congested, but only in the necrotizing form are severe hemorrhagic and necrotizing changes seen. The necrosis usually begins in the periphery of the gland with intrapancreatic and peripancreatic fat necrosis as a prominent feature (59,60). The severity of acute pancreatitis is determined by the state of preservation of the pancreatic capsule (47). The necrosis in man is considered to be a coagulative necrosis resulting from pancreatic infarction (1), although recently both coagulative and liquefactive necrosis have been demonstrated in pancreata of human origin (59,60). Activated pancreatic

enzymes are thought to be responsible for the pancreatic necrosis. The tissue damage effectuated by the digestive enzymes may be potentiated by ischemia (11). Arteriolar spasm, venous congestion, vasculitis and thrombosis of local blood vessels may be important determinants of the extent and severity of the disease (61).

There is experimental evidence that different agents lead to different morphological changes.

3.4.2. Proteolytic enzymes

Trypsin and chymotrypsin, injected retrogradely into the pancreatic duct, cause edema; at higher doses edema, hemorrhage and vascular lesions will be seen (4). The acinar tissue exhibits scattered liquefactive necrosis with abundant leukocyte infiltration. Trypsin is not thought to cause autodigestion in itself, whereas chymotrypsin may take part in the autodigestive process (4). In addition, other proteolytic enzymes such as elastase and kallikrein lead to vascular and capillary damage, but necrosis does not occur (4). Elastase dissolves elastic fibers and is said to play a major role in producing the vascular changes in the pancreas (5).

3.4.3. Lipolytic enzymes

The lipolytic enzymes lipase and phospholipase A have been held responsible for the severe necrosis in acute pancreatitis. Lipase, which is excreted in its active form, produces severe parenchymal necrosis when injected into the parenchyma (56,57). It has been postulated that when lipase reaches the intrapancreatic fat, fat necrosis develops with local accumulation of free fatty acids. The free fatty acids may exert a detergent effect on the cellular membranes with subsequent acinar necrosis (56,57).

Phospholipase A readily destroys cellular membranes by forming lysophospholipids, which are strongly cytotoxic. When phospholipase A (especially in combination with bile salts (39)) or lysolecithin is injected into the pancreatic duct in rats, severe parenchymal necrosis develops. The histomorphological pattern of the coagulation necrosis in the rat pancreas resembles that found for humans (38,39,62). Ischemia is thought to play an important role in the development of coagulation necrosis (39,63). The

lysolecithin content of the rat pancreas increases after the induction of acute pancreatitis (64), as has also been reported for human pancreata (1,38,39). The use of certain phospholipase A inhibitors is associated with less pancreatic necrosis (65). Phospholipase A2 is thought to be an ideal marker of acinar breakdown and most probably reflects the extent of tissue destruction (45). The transformation of lecithin into cytotoxic lysolecithin is believed to be an important step in the development of severe necrotizing pancreatitis from simple edematous forms (66). Phospholipase A2 activity may also lead to the formation of vasoactive mediators, such as the prostaglandins and leukotrienes, which can mediate ischemia by causing arteriolar spasms, venous congestion and thrombosis. Endogenous prostanoids may thus act as homeostatic agents that modulate the degree of cellular deterioration. Cell function can be further disturbed when these prostanoids, thromboxanes, leukotrienes and lysophospholipids are generated in situ (see Chapter 4). Ischemia is severe in the rat pancreas after intraductal injection of bile acids that cause acute pancreatitis (39,63). Trypsin is also activated in the ischemic gland and may in turn activate prophospholipase (39,67).

3.5. PANCREATIC ENZYMES AND SYSTEMIC MANIFESTATIONS OF PANCREATIC DISEASE

3.5.1. Introduction

Although acute pancreatitis begins with a local inflammation of the pancreas, widespread changes leading to multiple organ failure may be seen later in the course of the disease. The peritoneal cavity fills with a fluid that is rich in activated enzymes, histamine-releasing factors, vasoactive amines and other substances - all of which, when released into the systemic circulation, have a profound effect on myocardial function, vascular tone, endothelial integrity and/or complement activation (11,68). The peritoneal blood flow is increased during acute pancreatitis owing to the inflammatory effect of the peritoneal exudate (69). Activated enzymes may reach the circulation directly via the veins draining the pancreas and peripancreatic tissues or via the thoracic duct and absorption from the peritoneal cavity (15,70). Absorption from the peritoneal cavity was thought to be of major importance in one study (15), though not in another (70). Peritoneal lavage as well as intraperitoneal antiprotease therapy has been propagated as a means of

eliminating the toxic intraperitoneal pancreatic exudate (71-74). Some beneficial results have been reported in the experimental setting (75), though the efficacy of lavage has not been proven clinically (72).

Activated enzymes and other factors, when released into the systemic circulation, are presumed to be the mediators of many complications of pancreatitis. The concentration of proteolytic enzymes in the systemic circulation increases during acute pancreatitis. Usually the zymogen (inactive) form or a complex of active enzyme and inhibitor is seen (12,49,52). There is little direct evidence that free active proteases circulate in acute pancreatitis. In severe cases up to 30% of alpha 2-macroglobulin (the most important circulating antiprotease) is bound to trypsin. Plasma reserves of this inhibitor are never exhausted (49,76). However, it is suggested that the complexes of active enzyme and inhibitor retain the capacity to digest small peptide fragments, which can be mediators of complications (77).

Activation of the complement and kinin systems occurs in vitro at alpha 2-macroglobulin concentrations below 30% of normal (12,77); both complement and kinin activation cause profound systemic alterations and are closely related to the severity of the attack of pancreatitis (12,77).

The concentrations of the lipolytic enzymes also increase during acute pancreatitis (44,78-81). Increased serum phospholipase A2 activity correlates better with the severity of the disease than that of amylase, lipase or trypsin and is related to the prognosis in experimental and clinical situations (44,78,81). There are no known naturally occurring inhibitors of phospholipase A and lipase in man.

Pancreatic enzymes may play a role, directly or indirectly, in the following complications of acute pancreatitis:

a. cardiovascular disorders characterized by tachycardia, hypotension and severe shock, myocardial depression, capillary leak and hypovolemia,

b. respiratory insufficiency characterized by hypoxemia, alveolar membrane damage, pulmonary edema, alveolar-capillary leak and the acute respiratory distress syndrome,

c. renal failure,

d. fat necrosis found not only in peripancreatic tissue but also in subcutaneous tissues, synovial tissue, bone and the pericardium.

3.5.2. Proteolytic enzymes

It has been suggested that circulating proteolytic enzymes play a role in respiratory insufficiency and circulatory shock (11,82,83). There is evidence that elastase in particular affects the pulmonary vessels (84). Vasoactive peptides are released after activation of the proteases (78,85-87). By inducing hypovolemia, proteases cause renal hypoperfusion leading to acute renal failure (88). Proteolytic enzymes activate the different cascade systems of the body, such as the complement, kinin, coagulation and fibrinolytic systems (87). Both consumptive coagulopathy and increased fibrinolysis are seen in acute pancreatitis (89). The different cascade systems can interact with one another through activation of factor XII, the Hageman factor (12).

Autopsy studies of patients with acute pancreatitis have demonstrated the presence of fibrin and platelet microthrombi in pulmonary vessels (12,82,90,91). In addition thrombi have been found in the glomeruli (11). The basement membrane may also be damaged by circulating enzyme/enzyme inhibitor complexes (8).

Complement activation can lead to the aggregation of granulocytes and platelets, a pathogenetic factor of the sudden blindness sometimes seen in acute pancreatitis (Purtscher's syndrome)(92).

The clinical significance of the inhibition of the proteases in acute pancreatitis is not clear since in many trials enzyme inhibition did not have a significant effect on either survival or any other parameter (41-43).

3.5.3. Lipolytic enzymes

Lipase is held responsible for the fat necrosis. Disseminated fat necrosis could be reproduced experimentally when toxic ascitic fluid was infused intravenously (68). Lipase (as well as phospholipase A) is also thought to cause the encephalopathy due to demyelinization sometimes seen in acute pancreatitis (11,39). In addition, lipase may mediate the release of free fatty acids, especially in patients with acute pancreatitis due to associated hyperlipemia (11); free fatty acids may in turn cause pulmonary disorders (82,93,94).

Circulating phospholipase A, but also many other mechanisms, is presumed to

affect the alveolar membranes and pulmonary surfactant, leading in severe cases to the acute respiratory distress syndrome (39,82,95,96). Lecithin in the surfactant is a potential substrate for phospholipase activity. During experimental acute pancreatitis lysolecithin formation in pulmonary tissue increased (39,97,98). Intravenous phospholipase A infusions in dogs led to respiratory failure (39,96). Phospholipase A may be involved in the release of the cardioinhibitory factors produced in shock, such as the myocardial depressant factor (39,99). Phospholipase A also causes histamine release from mast cells in the presence of bile acids (4). Circulating phospholipase A2 contributes significantly to the hypotension associated with septic shock (100,101). Infusion of phospholipase A2 causes a decrease in renal perfusion that may lead to renal failure (91). The action of phospholipase A2 on the phospholipids in cell membranes leads to the liberation of arachidonic acid, which is the major precursor of eicosanoids (102). Eicosanoids are biologically very active mediators, which are thought to play a role in the pathogenesis and pathophysiology of several disorders, such as respiratory insufficiency, renal insufficiency and shock (102). Circulating cytoplasmic phospholipase A2 can directly stimulate de novo eicosanoid synthesis in healthy tissues (100, 103). Inhibition of phospholipase A2 will prevent the formation of eicosanoids. Inhibitors of phospholipase A2 activity have been administered experimentally and clinically with some success (64,65,95,104-109).

Inhibition of phospholipase A2 activity can be achieved by direct interaction with the enzyme, interference with binding to the substrate or interference with binding to calcium ions (39,110).

3.6. CONCLUSION

It will be clear from this chapter that at present a hypothetical pathogenesis that covers all aspects cannot be given. Further research is needed to clarify the intriguing background of acute necrotizing pancreatitis.

3.7. REFERENCES

1. Creutzfeldt W, Schmidt H. Aetiology and pathogenesis of pancreatitis (current concepts). Scand J Gastroent 1970;6(S):47-62.

2. Schiller WR, Suriyapa C, Anderson MC. Current research review. A review of experimental pancreatitis. J Surg Res 1974;16:69-90.

3. Kaplan MH. Stress, pancreatic perfusion, and acute pancreatitis: a unified concept pathogenesis. Mt Sinai J Med 1985;52:326-330.

4. Creutzfeldt W, Lankisch PG. Acute pancreatitis: Etiology and pathogenesis. in: Bockus' Gastroenterology, 4th edition, J.E. Berk, editor-in-chief, W.B. Saunders Company, London, 1985, pp. 3971-3992.

5. Davis conference: Geokas MC, moderator. Acute pancreatitis. Ann Intern Med 1985;103:86-100.

6. Seelig R, Seelig HP. The possible role of serum complement system in the formal pathogenesis of acute pancreatitis. II. Cobra venom factor pancreatitis - sodium taurocholate and deoxycholate pancreatitis. Acta Hepatogastroenterology 1975;22:335-346.

7. Seelig R, Lankisch PG, Koop H, et al. Complement system in sodium taurocholate pancreatitis in the rat. Res Exp Med 1978;174:57-65.

8. Steer ML. Etiology and pathophysiology of acute pancreatitis. In: The exocrine pancreas: biology, pathobiology, and diseases, edited by V.L.W. Go, et al. Raven Press, New York, 1986, pp. 465-474.

9. Steer ML, Meldolesi J, Figarella C. Pancreatitis. The role of lysosomes. Dig Dis Sci 1984;29:934-938.

10. Steer ML, Meldolesi J. The cell biology of experimental pancreatitis. N Engl J Med 1987;316:144-150.

11. Warshaw AL, Richter JM. A practical guide to pancreatitis. Curr Probl Surg 1984;21:7-15.

12. Lasson A. Acute pancreatitis in man. A clinical and biochemical study of pathophysiology and treatment. Scand J Gastroenterol 1984;99(S):1-57.

13. Warshaw AL, O'Hara PJ. Susceptibility of the pancreas to ischemic injury in shock. Ann Surg 1978;188:197-201.

14. Schiller WR, Anderson MC. Microcirculation of the normal and inflamed canine pancreas. Ann Surg 1975;181:466-470.

15. Lange JF. Experimental studies on bilesalt-induced acute pancreatitis in the rat. Thesis. University of Amsterdam. Amsterdam, 1987.

16. Broe PJ, Zuidema GD, Cameron JL. The role of ischemia in acute pancreatitis: studies with an isolated perfused canine pancreas. Surgery 1982;91:377-382.

17. Lefer AM, Spath JA jr. Pancreatic hypoperfusion and the production of a myocardial depressant factor. Ann Surg 1974;179:868-876.

18. Lefer AM. Minireview: The role of lysosomes in circulatory shock. Life Sci 1976;19:1803-1810.

19. Kivisaari L. The effect of experimental pancreatitis and diabetes on the microvasculature of the rat pancreas. Scand J Gastroent 1979;14:689-695.

20. Nuutinen P, Kivisaari L, Standertskjold-Nordenstam C-G, Lempinen M, Schroder T. Microangiography of the pancreas in experimental oedemic and haemorrhagic pancreatitis. Scand J Gastroenterol 1986;21(S26):12-17.

21. Aho HJ, Nevalainen TJ. Experimental pancreatitis in the rat: ultrastructure of sodium taurocholate-induced pancreatic lesions. Scand J Gastroent 1980;15:417-424.

22. Donahue PE, Akimoto H, Ferguson JL, Nyhus LM. Vasoactive drugs in acute pancreatitis. Arch Surg 1984;119:477-480.

23. Goodhead B. Acute pancreatitis and pancreatic blood flow. Surg Gyn Obstet 1969;69:331-340.

24. Goodhead B, Wright PW. The effect of postganglionic sympathectomy on the development of hemorrhagic pancreatitis in the dog. Ann Surg 1969;170:951-960.
25. Wright PW, Goodhead B. The value of dextrans in the treatment of experimental pancreatitis. Surgery 1970;67:807-815.
26. Crocket KV, Reising JR, Wirman JA, Gau N, Joffe SN. Effect of dextran 40 and aprotinin on experimental acute pancreatitis. J Surg Res 1984;36:256-264.
27. Sanfey H, Bulkley GB, Cameron JL. The pathogenesis of acute pancreatitis. The source and role of oxygen-derived free radicals in three different experimental models. Ann Surg 1985;201:633-639.
28. Sanfey H, Sarr MG, Bulkley GB, Cameron JL. Oxygen-derived free radicals and acute pancreatitis: a review. Acta Physiol Scand 1986;548(S):109-118.
29. Bulkley GB. Comment on: Lelcuk S, Alexander F, Kobzik L, et al. Prostacyclin and thromboxane A2 moderate postischemic renal failure. Surgery 1985;98:207-212, p 211.
30. Cuschieri A, Cumming JGR, Wood RAB, Baker PR. Evidence for sphincter dysfunction in patients with gallstone associated pancreatitis: effect of ceruletide in patients undergoing cholecystectomy for gallbladder disease and gallstone associated pancreatitis. Br J Surg 1984;71:885-888.
31. McCutcheon AD. A fresh approach to the pathogenesis of pancreatitis. Gut 1968;9:296-310.
32. Reber HA, Roberts C, Way LW. The pancreatic duct mucosal barrier. Am J Surg 1979;137:128-134.
33. Reber HA, Farmer RC, Maslin SC. Effect of bile on the permeability of the pancreatic duct to macromolecules. Gastroenterology 1982;82:1156.
34. Wedgwood KR, Adler G, Kern H, Reber HA. Effects of oral agents on pancreatic duct permeability. A model of acute alcoholic pancreatitis. Dig Dis Sci 1986;31:1081-1088.
35. Armstrong CP, Taylor TV, Torrance HB. Effects of bile, infection and pressure on pancreatic duct integrity. Br J Surg 1985;72:792-795.
36. Haboubi NY, Ali HH, Braganza JM. Altered liver histology in patients with pancreatitis: a clue to etiology? Mt Sinai J Med 1986;53:380-388.
37. Arnesjo B. The formation of lysolecithin in rat pancreas after experimentally induced bile salt pancreatitis. Acta Chir Scand 1971;137:351-354.
38. Schmidt H, Creutzfeldt W. The possible role of phospholipase A in the pathogenesis of acute pancreatitis. Scand J Gastroent 1969;4:39-48.
39. Nevalainen TJ. Review. The role of phospholipase A in acute pancreatitis. Scand J Gastroent 1980;15:641-650.
40. Whicher JT, Barnes MP, Brown A, et al. Complement activation and complement control proteins in acute pancreatitis. Gut 1982;23:944-950.
41. Millat B, Zazzo J-F, Poynard T. Les essais therapeutiques randomises dans le traitement de la pancreatite aigue. Gastroenterol Clin Biol 1986;10:341-354.
42. Lankisch PG. Acute and chronic pancreatitis. An update on management. Drugs 1984;28:554-564.
43. Cox AG. MRC multicentre trial. Morbidity of acute pancreatitis: the effect of aprotinin and glucagon. Gut 1980;21:334-339.
44. Nevalainen TJ, Eskola JU, Aho AJ, et al. Immunoreactive phospholipase A2 in serum in acute pancreatitis and pancreatic cancer. Clin Chem 1985;31:1116-1120.
45. Delk AS, Durie PR, Fletcher TS, Largman C. Radioimmunoassay of active pancreatic enzymes in sera from patients with acute pancreatitis. I. Active carboxypeptidase B. Clin Chem 1985;31:1294-1300.

46. McMahon MJ. Acute pancreatitis. in: Diseases of the gut and pancreas, edited by Misiewicz JJ, Pounder RE, Venables CW. Blackwell scientific publications, London, 1987, pp. 499-518.
47. Becker V. Pathological anatomy and pathogenesis of acute pancreatitis. World J Surg 1981;5:303-313.
48. Adham NF, Song MK. Haberfelde GC. Relationship between the functional status of the reticuloendothelial system and the outcome of experimentally induced pancreatitis in young mice. Gastroenterology 1983;84:461-469.
49. McMahon MJ, Bowen M, Mayer AD, Cooper EH. Relation of alpha 2-macroglobulin and other antiproteases to the clinical features of acute pancreatitis. Am J Surg 1984;147:164-170.
50. Drivas G, James O, Wardle N. Study of reticuloendothelial phagocytic capacity in patients with cholestasis. Br Med J 1976;1:1568-1569.
51. Balldin G, Ohlsson K. Demonstration of pancreatic protease-antiprotease complexes in the peritoneal fluid of patients with acute pancreatitis. Surgery 1979;85:451-456.
52. Sanfey H, Bulkley GB, Cameron JL. The role of oxygen-derived free radicals in the pathogenesis of acute pancreatitis. Ann Surg 1984;200:405-413.
53. Sarr MG, Bulkley GB, Cameron JL. The role of leukocytes in the production of oxygen-derived free radicals in acute experimental pancreatitis. Surgery 1987;101:292-296.
54. Hatao M. On etiology and pathophysiology of acute pancreatitis with special reference to participation of phospholipase A. Arch Jap Chir 1969;38:76-106.
55. Zieve L, Vogel WC. Measurement of lecithinase A in serum and other body fluids. J Lab Clin Med 1961;57:586-599.
56. Schmidt H, Lankisch PG. Fat necrosis - a cause of pancreatic parenchymal necrosis. Digestion 1978;17:84-91.
57. Schmidt-Moormann P, Boger A. Tissue damage by fatty acids released by lipolysis. Path Res Pract 1981;171:303-313.
58. Kloppel G, Dreyer T, Willemer S, Kern HF, Adler G. Human acute pancreatitis: its pathogenesis in the light of immunocytochemical and ultrastructural findings in acinar cells. Virchows Arch ?Pathol Anat? 1986;409:791-803.
59. Phat VN, Guerrieri MT, Alexandre JH, Camilleri JP. Early histological changes in acute necrotizing hemorrhagic pancreatitis. A retrospective pathological study of 20 total pancreatectomy specimens. Path Res Pract 1984;178:273-279.
60. Nordback I, Lauslahti K. Clinical pathology of acute necrotising pancreatitis. J Clin Pathol 1986;39:68-74.
61. Rao SS, Watt IA, Donaldson LA, Crocket A, Joffe SN. A serial histologic study of the development and progression of acute pancreatitis in the rat. Am J Pathol 1982;103:39-46.
62. Aho HJ, Nevalainen TJ. Experimental pancreatitis in the rat. Light and electron microscopical observations on early pancreatic lesions induced by intraductal injection of trypsin, phospholipase A2, lysolecithin and non-ionic detergent. Virchows Arch (Cell Pathol) 1982;40:347-356.
63. Aho HJ, Nevalainen TJ, Aho AJ. Experimental pancreatitis in the rat. Development of pancreatic necrosis, ischemia and edema after intraductal sodium taurocholate injection. Eur Surg Res 1983;15:28-36.
64. Aho HJ, Nevalainen TJ, Lindberg RLP, Aho AJ. Experimental pancreatitis in the rat. The role of phospholipase A in sodium taurocholate-induced acute haemorrhagic pancreatitis. Scand J Gastroent 1980;15:1027-1031.
65. Schroder T, Lempinen M, Nordling S, Kinnunen PKJ. Chlorpromazine treatment of experimental acute fulminant pancreatitis in pigs. Eur Surg Res

1981;13:143-151.
66. Freise J, Schmidt FW, Magerstedt P, Schmid K. Gabexate mesilate and camostate: new inhibitors of phospholipase A2 and their influence on the alpha-amylase activity in serum of patients with acute pancreatitis. Clin Biochem 1985;18:224-229.
67. Wanke M. Role of proteases in the development of acute pancreatitis. Adv Exp Med Biol 1984;167:463-475.
68. Takada Y, Appert HE, Howard JM. Vascular permeability induced by pancreatic exudate formed during acute pancreatitis in dogs. Surg Gyn Obstet 1976;143:779-783.
69. Lehtola A. Peritoneal blood flow during acute experimental pancreatitis. Scand J Gastroenterol 1986;21:756-760.
70. Mayer AD, Airey M, Hodgson J, McMahon MJ. Enzyme transfer from pancreas to plasma during acute pancreatitis. The contribution of ascitic fluid and lymphatic drainage of the pancreas. Gut 1985;26:876-881.
71. Editorial. Changing concepts in the evaluation and treatment of acute severe pancreatitis. Intensive Care Med 1985;11:107-109.
72. Pellegrini CA. The treatment of acute pancreatitis: a continuing challenge. N Engl J Med 1985;312:436-438.
73. Mayer AD, McMahon MJ, Corfield AP, et al. Controlled clinical trial of peritoneal lavage for the treatment of severe acute pancreatitis. N Engl J Med 1985;312:399-404.
74. Imrie CW. Leading article. Peritoneal lavage in severe acute pancreatitis. Br J Surg 1985;72:677.
75. Satake K, Koh I, Nishiwaki H, Umeyama K. Toxic products in hemorrhagic ascitic fluid generated during experimental acute hemorrhagic pancreatitis in dogs and a treatment which reduces their effect. Digestion 1985;32:99-105.
76. Goodman AJ, Bird NC, Johnson AG. Antiprotease capacity in acute pancreatitis. Br J Surg 1986;73:796-798.
77. Lasson A, Ohlsson K. On the potential role of trypsin and trypsin inhibitors in acute pancreatitis. Adv Exp Med Biol 1984;167:477-487.
78. Schroder T, Kivilaakso E, Kinnunen PKJ, Lempinen M. Serum phospholipase A2 in human acute pancreatitis. Scand J Gastroent 1980;15:633-636.
79. Mero M, Schroder T, Tenhunen R, Lempinen M. Serum phospholipase A2, immunoreactive trypsin, and trypsin inhibitors during human acute pancreatitis. Scand J Gastroent 1982;17:413-416.
80. Schroder T, Somerharju P, Lempinen M. Phospholipase A2 in serum and ascitic exudate in experimental acute hemorrhagic pancreatitis in pigs. Eur Surg Res 1979;11:172-178.
81. Mizuma K, Schroder T, Kaarne M, Korpela H, Lempinen M. Serum phospholipase A2 in diet-induced pancreatitis. Eur Surg Res 1984;16:156-161.
82. Malik AB. Review article. Pulmonary edema after pancreatitis. Role of humoral factors. Circ Shock 1983;10:71-80.
83. Garcia-Szabo RR, Malik AB. Pancreatitis-induced increase in lung vascular permeability. Protective effect of Trasylol. Am Rev Respir Dis 1984;129:580-583.
84. Lungarella G, Gardi C, De Santi MG, Luzi P. Pulmonary vascular injury in pancreatitis: evidence for a major role played by pancreatic elastase. Exp Mol Pathol 1985;42:44-59.
85. Katz W, Silverstein M, Kobold EE, Thal AP. Trypsin release, kinin production and shock. Arch Surg 1964;89:322-331.
86. Lefer AM. The pathophysiologic role of myocardial depressant factor as a mediator of circulatory shock. Klin Wochenschr 1982;60:713-716.
87. Lasson A. Review. Acute pancreatitis. The correlation between clinical course, protease inhibitors, and complement and kinin activation. Scand J

Gastroenterol 1984;19:707-710.
88. Levy M, Geller R, Hymovitch S. Renal failure in dogs with experimental acute pancreatitis: role of hypovolemia. Am J Physiol 1986;251:(F)969-977.
89. Lasson A, Ohlsson K. Consumptive coagulopathy, fibrinolysis and protease-antiprotease interactions during acute human pancreatitis. Thromb Res 1986;41:167-183.
90. Satake K, Uchima K, Umeyama K, Appert HE, Howard JM. The effects upon blood coagulation in dogs of experimentally induced pancreatitis and the infusion of pancreatic juice. Surg Gyn Obstet 1981;153:341-345.
91. Berry AR, Davies GC, Millar AM, Taylor TV. Changes in the biophysical properties and ultrastructure of lungs, and intrapulmonary fibrin deposition in experimental acute pancreatitis. Gut 1983;24:929-934.
92. Jacob HS, Craddock PR, Hammerschmidt DE, Moldow CF. Complement-induced granulocyte aggregation; an unsuspected mechanism of disease. N Engl J Med 1980;302:789-794.
93. Kimura T, Toung JK, Margolis S, Permutt S, Cameron JL. Respiratory failure in acute pancreatitis. A possible role for triglycerides. Ann Surg 1979;189:509-514.
94. Kimura T, Toung JK, Margolis S, Bell WR, Cameron JL. Respiratory failure in acute pancreatitis: the role of free fatty acids. Surgery 1980;87:509-513.
95. Tykka H, Mahlberg K, Pantzar P, Tallberg T. Phospholipase A2 inhibitors and their possible clinical use in the treatment of acute pancreatitis. Scand J Gastroent 1980;15:519-528.
96. Morgan AP, Jenny ME, Haessler H. Phospholipids, acute pancreatitis and the lungs: Effect of lecithinase infusion on pulmonary surface activity in dogs. Ann Surg 1968;167:329-335.
97. Idegami K, Mori K, Misumi A, Akagi M. Changes of alveolar stability and phospholipids in pulmonary surfactant in acute pancreatitis. Jpn J Surg 1983;13:227-235.
98. Aho HJ, Ahola RA, Tolvanen AM, Nevalainen TJ. Experimental pancreatitis in the rat. Changes in pulmonary phospholipids during sodium taurocholate-induced acute pancreatitis. Res Exp Med 1983;182:79-84.
99. Lefer AM. Properties of cardioinhibitory factors produced in shock. Fed Proc 1978;37:2734-2740.
100. Vincent JE. The use of the determination of phospholipase A2 in the diagnosis of pancreatitis, endotoxin and septic shock and inflammatory disorders. Report Erasmus University. Rotterdam, 1986.
101. Vadas P, Hay JB. Involvement of circulating phospholipase A2 in the pathogenesis of the hemodynamic changes in endotoxin shock. Can J Physiol Pharmacol 1983;61:561-566.
102. Ouwendijk RJT. Eicosanoids, endotoxins and liver disease. Thesis. Erasmus University. Rotterdam, 1985.
103. Flynn JT, Henry JM, Perkowski S. Phospholipase A2 stimulated release of prostanoids from the isolated, perfused rabbit liver: implications in regional cellular injury. Can J Physiol Pharmacol 1981;59:1268-1273.
104. Tykka HT, Vaittinen EJ, Mahlberg KL, et al. A randomized double-blind study using CaNa2EDTA, a phospholipase A2 inhibitor, in the management of human acute pancreatitis. Scand J Gastroenterol 1985;20:5-12.
105. Freise J, Melzer P, Schmidt FW, Horbach L. Gabexat mesilat in der behandlung der akuten pankreatitis. Ergebnisse der Hannoverschen multizentrischen doppelblindstudie mit 50 patienten. Z Gastroenterol 1986;24:200-211.
106. Schroder T, Kinnunen PKJ, Lempinen M. Xylocaine treatment in experimental pancreatitis in pigs. Scand J Gastroent 1978;13:863-865.

107. Freise J. Therapie der akuten pankreatitis mit dem synthetischen proteasen- und phospholipase A2-inhibitor gabexat mesilat. Fortschr Med 1983;101:1432-1436.

108. Kahle M, Klein U, Konig W, Martin U, Alles JU. Phospholipase A2-inhibitoren bei hamorrhagisch-nekrotisierender pankreatitis. Tierexperimentelle uberprufung eines neuen behandlungskonzeptes. Z Gastroenterol 1985;23:687-693.

109. Zoch G, Roth E, Holbling N, et al. Effekt von p-Bromophenacylbromid, einem phospholipase A-hemmer, bei experimenteller akuter pankreatitis der ratte. Res Exp Med 1984;184:179-189.

110. Blackwell GJ, Flower RJ. Inhibition of phospholipase. Br Med Bull 1983;39:260-264.

CHAPTER 4

PROSTAGLANDINS, THROMBOXANES AND THE PANCREAS.

4.1. INTRODUCTION

Eicosanoids (prostaglandins, thromboxanes and leukotrienes) are biologically active substances that are present, though not stored, in most mammalian cells and tissues. Biosynthesis, which immediately precedes release, takes place as follows: essential fatty acids, found mainly in the phospholipids of cell membranes, are liberated by several phospholipases, the most important being phospholipase A2. Arachidonic acid, the most abundant essential fatty acid, is transformed by the enzyme system cyclooxygenase to prostaglandins and thromboxanes and by another system (5-lipoxygenase) to leukotrienes. Leukotrienes are biologically active compounds that affect bronchial and vascular tone and cardiac function (1,2).

The action of the cyclooxygenases on arachidonic acid leads to the formation of the endoperoxides prostaglandin G2 and prostaglandin H2. These endoperoxides, which are unstable, are converted into the prostaglandins D2, E2, F2alpha and I2 as well as thromboxane A2 and some other compounds. Inactivation of the prostaglandins may occur rapidly in the lungs, liver, spleen, kidney and blood vessels. In vivo thromboxane A2 is converted to the inactive metabolite thromboxane B2. Prostaglandin I2 (prostacyclin) is metabolized mainly in the liver and degrades to 6-keto-prostaglandin F1alpha, which is biologically less active (1). Prostacyclin therefore escapes the pulmonary inactivation process, so that potentially it can become a circulating hormone (3).

The prostaglandins and thromboxanes affect almost every biological system, sometimes in different directions, and are thought to play an important role in the pathogenesis and pathophysiology of many diseases (1,4-8). The biological properties of these substances have been described extensively in several recent reviews (1,7). In the gastrointestinal tract prostaglandins are active at several levels, since they play a role in gastric acid secretion, peptic ulcer disease, cytoprotection and inflammatory bowel disease (1,4,8). Their precise function in the pathophysiology of gastrointestinal disorders has not yet been clarified, however. Although the actions of the

prostaglandins on the stomach have been studied extensively, their effects on the pancreas and pancreatic disease are mainly unknown and what is known is based exclusively on experimental data.

4.2. EICOSANOIDS AND THE NORMAL PANCREAS

4.2.1. Effects on blood flow

Prostaglandins I2 and E2 are dilators of the mesenteric vascular bed. They increase blood flow and decrease vascular resistance (9). In anesthesized dogs these prostaglandins increase pancreatic blood flow, whereas thromboxane A2 does not (10). Prostaglandins E2 and I2 are thought to contribute in particular to the maintenance of vascular tone in (at least) the canine pancreas (9). This concept is reinforced by the finding that indomethacin pretreatment decreased basal blood flow in the pancreas (11), although part of this effect must be ascribed to the anesthesia used in the experiments (12). In contrast to prostaglandins E2 and I2, thromboxane A2 is unlikely to be involved in the physiological regulation of normal tone in most vascular beds (13).

4.2.2. Effects on the pancreatic duct

The strong 16,16-dimethyl analogue of prostaglandin E2, when administered intravenously, prevents the permeability changes in the cat pancreatic duct induced by bile salts, aspirin and ethanol (14,15) in a dose-related manner. However, prostaglandin E2, prostaglandin I2 and indomethacin had no effect on deoxycholic acid-induced damage to the rat bilio-pancreatic duct (16). The property of prostaglandin E2 (and prostaglandin I2) that prevents permeability changes in the pancreatic duct has been attributed to "cytoprotection".

Cytoprotection is the property of certain prostaglandins that protects mucosal tissue located beneath the surface epithelium against hemorrhage and necrosis, when this mucosa is exposed to noxious agents (17). The damage to the pancreatic duct that may occur in acute pancreatitis, when bile salts and other detergents enter the duct system, may possibly be ascribed to an endogenous deficit in cytoprotective prostaglandins or a prostanoid imbalance. The prostaglandins I2 and E2 are inhibitors of pancreatic secretion (7,18),

like glucagon, calcitonin and somatostatin (19-22). Glucagon and calcitonin have been used to treat acute pancreatitis with unconvincing success (20,21). Somatostatin also has cytoprotective properties, perhaps via the prostaglandin system (23,24).

4.3. EICOSANOIDS AND THE INFLAMED PANCREAS

Eicosanoids are thought to be formed in acute pancreatitis. The pancreas is especially rich in phospholipase A2, and release of phospholipase A2 may be responsible for the eicosanoid production (7,25). Glazer and Bennett (26) demonstrated high levels of prostaglandin E-like activity in pancreatic venous blood in the early stages of acute pancreatitis in dogs.

4.3.1. Are eicosanoids locally active?

Eicosanoids may act as homeostatic agents that modulate the severity of damage to pancreatic cells. Thromboxane A2 and prostaglandin I2 exert opposing pharmacological actions on, for example, platelet function, vascular smooth muscle and gastrointestinal integrity (1). The synthesis of thromboxane A2 can be blocked by prostaglandin I2 (1). The imbalance between these two compounds is thought to result in dysfunction of cells, tissues and organs (3,27,28). Thromboxane A2 is a strong vasoconstrictor and induces platelet aggregation, whereas prostaglandin I2 does the opposite (27,28). Thromboxane A2 is a mediator of ischemia, possibly by activating the formation of free radicals (29). The eicosanoid cascade can both activate and be activated by the generation of free radicals (30). Postischemic renal failure is associated with a rise in the level of thromboxane B2 while the 6-keto-PGF1alpha levels remain stable; a thromboxane A2/prostacyclin ratio in favor of prostacyclin prevents the development of ischemia (29). Thromboxane A2, by its actions, decreases the microcirculation, while prostacyclin increases microcirculatory blood flow (8,28,31). Ischemia is believed to be an important event in the progression of pancreatic edema to necrosis (see Chapter 3). Eicosanoids may mediate many of the changes arising from ischemia. Ischemic splanchnic injury was prevented in rats when prostaglandin E2 was given (32).

4.3.2. Are eicosanoids systemically active?

Prostaglandins are found in the pancreatic exudate in acute pancreatitis (26,33) and may thus also enter the systemic circulation. However, no increase in prostaglandin-like activity was found in either arterial or mixed venous blood taken from dogs with acute pancreatitis (26). Little data is available on eicosanoid activity in the systemic circulation. Farias et al. (33) found elevated levels of thromboxane B2 and 6-keto-PGF1alpha in arterial and venous blood 5 hours after the induction of acute pancreatitis in pigs. Jacobs et al. (34) could not find any changes in plasma thromboxane B2 , 6-keto-PGF1alpha and prostaglandin E2 concentrations in dogs with acute pancreatitis, but their detection level for thromboxane B2 was high (650 pg/ml) and the time of sampling late (24 hours).

4.4. SOURCE OF EICOSANOIDS

Like activated pancreatic enzymes eicosanoids may enter the systemic circulation via several routes (see Chapter 3). Absorption of eicosanoids from ascitic fluid in the peritoneal cavity may occur; this has, for example, been implicated as the cause of several complications of liver cirrhosis, such as renal dysfunction, shock and coagulation disorders (1).

In addition to absorption, circulating phospholipase A2 of pancreatic origin can directly stimulate de novo eicosanoid synthesis in healthy tissues (35).

4.5. POSSIBLE SYSTEMIC ACTIONS OF EICOSANOIDS IN ACUTE PANCREATITIS

Considerable experimental data has been accumulated concerning the role of eicosanoids in endotoxic shock. Endotoxic shock is frequently seen in severe cases of acute pancreatitis (36). In endotoxic shock, thromboxane B2 levels are elevated in animals (37,38) as well as in man (39), whereas only slightly elevated or even depressed 6-keto-PGF1alpha levels are found (40,41). Inhibition of thromboxane A2 synthesis (38) as well as administration of exogenous prostaglandin I2 (42) appeared to have a positive effect on survival.

Prostaglandins E1 and I2 prevent the formation of the myocardial depressant factor, which plays a role in the cardioinhibitory changes in acute

pancreatitis (43,44).

Prostaglandins modulate the activity of the reticuloendothelial system (45,46). The reticuloendothelial system is thought to play an important role in acute pancreatitis, because it is responsible for the clearance of, for example, the alpha 2-macroglobulin-protease complexes (see Chapter 3).

The generation of cyclooxygenase products has been implicated as an important factor in pulmonary dysfunction (1). Thromboxane A2 and prostaglandin I2 are the main products in the lung. Pulmonary dysfunction occurs in acute pancreatitis. Thromboxane A2 plays a role in the alteration of lung mechanics and mediates pulmonary vasoconstriction and platelet sequestration following endotoxin infusions, but is not involved in changes in pulmonary vascular permeability (47). In acute pancreatitis pulmonary lysosomes (48), as well as renal (49) and hepatic lysosomes (50), are labile. Prostacyclin is able to stabilize the lysosomes, probably via a cytoprotective effect (28,49,50).

4.6. PROSTAGLANDINS FOR THE TREATMENT OF ACUTE PANCREATITIS

Because of the cytoprotective and other effects of prostaglandins in various organ systems, a number of attempts have been made to protect against acute pancreatitis in animal models. The administration of prostaglandin E series has yielded contradictory results: some authors report a protective effect with decreased mortality (51-53); other investigators found no demonstrable changes (54-56), while still others observed deleterious effects (57). The discrepancies are partly attributable to the experimental protocol employed (46). Prostacyclin has not been used so far to treat acute pancreatitis, because it is difficult to administer (58,59) and has dose-limiting cardiovascular effects. Recently, a more stable prostacyclin derivative with fewer adverse hemodynamic effects was developed (iloprost, ZK 36374) (59,60).

4.7. INHIBITORS OF EICOSANOID SYNTHESIS

The possible role of eicosanoids in many diseases has led to the development of inhibitors of their synthesis. Eicosanoid synthesis can be inhibited at several levels:

a. inhibition of phospholipase A2

b. inhibition of the cyclooxygenases

c. inhibition of the lipoxygenases

d. specific inhibition of, for example, thromboxane A2 synthesis

a. Many inhibitors of phospholipase A2 exist, including chloroquine (61). Phospholipase A2 is highly dependent on intracellular calcium. Since intracellular calcium is thought to be a major activator of phospholipase A2, it is probably a major factor controling arachidonate liberation (1,61). Calcium entry blockers, such as flunarizine and diltiazem, may therefore interfere with phospholipase A2; they are known to antagonize thromboxane A2 as well (62,63). Corticosteroids inhibit phospholipase A2 action by inducing the formation of lipocortin, an inhibitory protein (64).

b. Indomethacin and many other non-steroidal antiinflammatory drugs are known to be inhibitors of the cyclooxygenase systems (65).

c. The synthesis of leukotrienes can be inhibited by, for example, FPL 55712, an inhibitor of the 5-lipoxygenase pathway (66).

d. Thromboxane A2 synthetase blockers include dazoxiben (UK 37248) (67-69) and dazmegrel (UK 38485) (70).

Several biological effects mediated by prostaglandins and thromboxanes can be and have been studied by using blockers of the eicosanoid pathway. However, in many instances, further studies need to be performed to ensure that the changes observed are not merely epiphenomena.

4.8. CONCLUSION

It is possible that eicosanoids mediate much of the damage to pancreatic cells seen in acute necrotizing pancreatitis. They may also be responsible for symptoms of respiratory insufficiency, renal insufficiency and shock. Therefore it is interesting to investigate whether eicosanoids are formed during acute pancreatitis and to determine whether inhibition of eicosanoid synthesis is important for survival in acute necrotizing pancreatitis. Because certain eicosanoids (for example, thromboxane A2 and prostaglandin I2) affect biological systems in different directions it is also interesting to observe whether the specific balance between the eicosanoids is important for survival in acute necrotizing pancreatitis.

4.9. REFERENCES

1. Ouwendijk RJT. Eicosanoids, endotoxins and liver disease. Thesis. Erasmus University. Rotterdam, 1985.
2. Feuerstein G. Leukotrienes and the cardiovascular system. Prostaglandins 1984;27:781-802.
3. Moncada S. Biological importance of prostacyclin. Br J Pharmacol 1982;76:3-31.
4. Wilson DE, Kaymakcalan H. Prostaglandins: Gastrointestinal effects and peptic ulcer disease. Med Clin North Am 1981;65:773-787.
5. Hyman AL, Mathe AA, Lippton HL, Kadowitz PJ. Prostaglandins and the lung. Med Clin North Am 1981;65:789-808.
6. Nasjletti A, Malik KU. Interrelationships among prostaglandins and vasoactive substances. Med Clin North Am 1981;65:881-889.
7. Goerig M, Habenicht AJR, Schettler G. Eicosanoide und phospholipasen. Klin Wochenschr 1985;63:293-311.
8. Van Essen HA. The stress ulcer syndrome. Thesis. Erasmus University. Rotterdam, 1986.
9. Houvenaghel A, Schrauwer E, Wechsun L. Influence of primary prostaglandins, prostacyclin, and arachidonic acid on mesenteric hemodynamics in the pig. Prostaglandins Med 1979;2:83-95.
10. Homma T, Malik KU. Effect of prostaglandins on pancreatic circulation in anesthetized dogs. J Pharmacol Exp Ther 1982;222:623-628.
11. Studley JGN, Lee JB, Schenk WG. Pathophysiology of acute pancreatitis: evaluation of the effects and mode of action of indomethacin in experimental pancreatitis in dogs. J Surg Res 1982;32:563-568.
12. Studley JGN, Lee JB, Schenk WG. Effects of indomethacin on bloodflow in the normal pancreas in conscious dogs. J Surg Res 1984;37:464-466.
13. Whittle BJR, Moncada S. Pharmacological interactions between prostacyclin and thromboxanes. Br Med Bull 1983;39:232-238.
14. Reber HA, Tweedie JH, Mosley JG. The cytoprotective effect of 16,16-dimethyl-prostaglandin E2 on bile salt induced damage to the pancreas. Gastroenterology 1980;78:1241.
15. Tweedie JH, Mosley JG, Austin JL, Reber HA. Effect of 16,16-dimethyl-prostaglandin E2 on aspirin-induced permeability changes in the pancreatic duct. Am J Surg 1981;141:22-27.
16. Olazabal A. Effect of prostaglandins E2 and I2 and of indomethacin on deoxycholic acid-induced damage to the rat bile-pancreatic duct. Gastroenterology 1983;84:928-34.
17. Robert A, Lancaster C, Davis JP, et al. Cytoprotection by prostaglandin occur in spite of penetration of absolute ethanol into the gastric mucosa. Gastroenterology 1985;88:328-333.
18. Konturek SJ, Tasler J, Jaworek J, et al. Prostacyclin inhibits pancreatic secretion. Am J Physiol 1980;238:(G)531-536.
19. Lamberts SWJ. Non pituitary actions of somatostatin. A review on the therapeutic role of SMS 201-995 (Sandostatin). Report Erasmus University. Rotterdam, 1987.
20. Durr GHK. Therapeutic trials in acute pancreatitis. German experience. Gastroenterologie Clinique et Biologique 1978;11:954.
21. Imrie CW, Blumgart LH. Glucagon therapy in acute pancreatitis. Br Med J 1974;1:38.
22. Dollinger HC, Raptis S, Pfeiffer EF. Effects of somatostatin on exocrine and endocrine pancreatic function stimulated by intestinal hormones in man. Horm Metab Res 1976;8:74-78.

23. Szabo S, Usadel KH. Cytoprotection-organoprotection by somatostatin; gastric and hepatic lesions. Experientia 1982;38:254-256.
24. Baxter JN, Jenkins SA, Day DW, et al. Effects of somatostatin and a long-acting somatostatin analogue on the prevention and treatment of experimentally induced acute pancreatitis in the rat. Br J Surg 1985;71:382-385.
25. Vogt W. Role of phospholipase A2 in prostaglandin formation. Adv Prostaglandins Thromboxane Res 1978;3:89-95.
26. Glazer G, Bennett A. Prostaglandin release in canine acute hemorrhagic pancreatitis. Gut 1976;17:22-26.
27. Bunting S, Moncada S, Vane JR. The prostacyclin-thromboxane A2 balance: pathophysiological and therapeutic implications. Br Med Bull 1983;39:271-276.
28. Moncada S, Vane JR. Prostacyclin and its clinical applications. Ann Clin Res 1984;16:241-252.
29. Lelcuk S, Alexander F, Kobzik L, et al. Prostacyclin and thromboxane A2 moderate postischemic renal failure. Surgery 1985;98:207-212.
30. Bulkley GB. Comment on: Lelcuk S, Alexander F, Kobzik L, et al. Prostacyclin and thromboxane A2 moderate postischemic renal failure. Surgery 1985;98:207-212, p 211.
31. Moncada S, Vane JR. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A2 and prostacyclin. Pharmacol Rev 1979;30:293-332.
32. Dawiskiba J, Isaksson B, Jeppson B, Hagerstrand I, Bengmark S. Cytoprotective effect of PGE2 on ischemic splanchnic injuries in the rat. Eur Surg Res 1984;16:77-83.
33. Farias LR, Frey CF, Holcroft JW, Gunther R. Effect of prostaglandin blockers on ascites fluid in pancreatitis. Surgery 1985;98:571-578.
34. Jacobs RM, Murtaugh RJ, Fertel RH. Platelet function in experimentally induced pancreatitis in the dog. Thromb Haemostat 1986;55:197-200.
35. Flynn JT, Henry JM, Perkowski S. Phospholipase A2 stimulated release of prostanoids from the isolated, perfused rabbit liver: implications in regional cellular injury. Can J Physiol Pharmacol 1981;59:1268-1273.
36. Fine J. Acute pancreatitis. Lancet 1975;1:1092.
37. Cook JA, Wise WC, Halushka PV. Elevated thromboxane levels in the rat during endotoxic shock. J Clin Invest 1980;65:227-230.
38. Wise WC, Cook JA, Halushka PV, Knapp DR. Protective effects of thromboxane synthethase inhibitors in endotoxic shock. Circ Res 1980;46:854-859.
39. Reines HD, Halushka PV, Cook JA, Wise WC, Rambo W. Plasma thromboxane concentrations are raised in patients dying with septic shock. Lancet 1982;2:174-175.
40. Webb PJ, Westwick J, Scully MF, et al. Do prostacyclin and thromboxane play a role in endotoxic shock? Br J Surg 1981;68:720-724.
41. Carmona RH, Tsao TC, Trunkey DD. The role of prostacyclin and thromboxane in sepsis and septic shock. Arch Surg 1984;119:189-192.
42. Fletcher JR, Ramwell PW. The effects of prostacyclin (PGI2) on endotoxin shock and endotoxin-induced platelet aggregation in dogs. Circ Shock 1980;7:299-308.
43. Lefer AM, Tabas J, Smith EF. Salutary effects of prostacyclin in endotoxic shock. Pharmacology 1980;21:206-212.
44. Lefer AM. The pathophysiologic role of myocardial depressant factor as a mediator of circulatory shock. Klin Wochenschr 1982;60:713-716.
45. Reiko T, Werb Z. Secretory products of macrophages and their physiological functions. Am J Physiol 1984;246:(C)1-6.
46. Taylor IL, Coelle EF, Adham NF. Factors that modify the effects of prostaglandin on diet-induced acute pancreatitis in mice. Scand J

Gastroenterol 1985;20(S112):101-105.
47. Brigham KL, Ogletree M, Snapper J, Hinson J, Parker R. Prostaglandins and lung injury. Chest 1983;83(S):70-72.
48. Wereszczynska U, Dlugosz J, Gabryelewicz A, Andrzejewska A. The lung lysosomal hydrolases and phospholipases in acute experimental pancreatitis with reference to heparin treatment. Path Res Pract 1986;181:576-585.
49. Triebling AT, Dlugosz J, Brzozowski J, et al. The renal lysosomes in acute experimental pancreatitis in dogs treated with prostacyclin (PGI2). Path Res Pract 1984;178:280-288.
50. Dlugosz J, Gabryelewicz A, Andrzejewska A, et al. Prostacyclin (PGI2) stabilizes hepatic lysosomes during acute experimental pancreatitis in dogs. Z Exp Chir 1982;15:210-218.
51. Manabe T, Steer ML. Protective effects of PGE2 on diet-induced acute pancreatitis in mice. Gastroenterology 1980;78:777-781.
52. Coelle EF, Adham N, Elashoff J, Lewin K, Taylor IL. Effects of prostaglandin and indomethacin on diet-induced acute pancreatitis in mice. Gastroenterology 1983;85:1307-1312.
53. Standfield NJ, Kakkar VV. Prostaglandins and acute pancreatitis - experimental and clinical studies. Br J Surg 1983;70:573-576.
54. Lankisch PG, Goke B, Kunze H, Otto J, Winckler K. Does PGE2 have a beneficial effect on acute experimental pancreatitis in the rat? Hepatogastroenterology 1983;36:148-150.
55. Martin DM, Someren AO, Nasrallah SM. The value of prostaglandin E2 in ethionine induced pancreatitis. Gastroenterology 1980;78:1241.
56. Crocket KV, Brackett K, Crocket A, et al. Prostaglandin E1 treatment in experimental acute pancreatitis in the rat. Eur Surg Res 1984;16:265-73.
57. Wedgwood KR, Farmer RC, Reber HA. A model of hemorrhagic pancreatitis in cats - role of 16,16-dimethyl prostaglandin E2. Gastroenterology 1986;90:32-39.
58. Lewis PJ, Dollery CT. Clinical pharmacology and potential of prostacyclin. Br Med Bull 1983;39:281-284.
59. Cowley AJ, Heptinstall S, Hampton JR. Effects of prostacyclin and of stable prostacyclin analogue ZK 36374 on forearm blood flow and blood platelet behaviour in man. Thromb Haemostat 1985;53:90-94.
60. Krause W, Beckmann, Schubert M. Pharmacokinetics and pharmacodynamics of the stable prostacyclin analogue ZK 36374 in the cat. Prostaglandins Leukotrienes Medicine 1984;15:187-197.
61. Nevalainen TJ. The role of phospholipase A in acute pancreatitis. Scand J Gastroent 1980;15:641-650.
62. De Clerck F, Van Nueten JM. Platelet-mediated vascular contraction. Inhibition by flunarizine, a calcium entry blocker. Bioch Pharmacol 1983;32:765-771.
63. Mehta J, Mehta P, Ostrowski N. Calcium blocker diltiazem inhibits platelet activation and stimulates vascular prostacyclin synthesis. Am J Med Sci 1986;291:21-24.
64. Russo-Marie F. La lipocortine, proteine de controle de l'activation membranaire dans la reaction inflammatoire. Pathol Biol (Paris) 1986;34:227-233.
65. Higgs GA, Vane JR. Inhibition of cyclo-oxygenase and lipoxygenase. Br Med Bull 1983;39:265-270.
66. Augstein J, Farmer JB, Lee TB, Sheard P, Tattersall ML. Selective inhibitor of slow reacting substance of anaphylaxis. Nature New Biol 1973;245:215-217.
67. Verstraete M. Introduction: thromboxane in biological systems and the possible impact of its inhibition. Br J Clin Pharmacol 1983;15(S):7-11.

68. Tyler HM. Dazoxiben: a pharmacological tool or clinical candidate? Br J Clin Pharmacol 1983;15(S):13-16.

69. O'Keefe EH, Liu ECK, Greenberg R, Ogletree ML. Effects of a thromboxane synthetase inhibitor and a thromboxane antagonist on release and activity of thromboxane A2 and prostacyclin in vitro. Prostaglandins 1985;29:785-797.

70. Fischer S, Struppler M, Bohlig B, et al. The influence of selective thromboxane synthetase inhibition with a novel imidazol derivative, UK - 38485, on prostanoid formation in man. Circulation 1983;68:821-826.

CHAPTER 5

A MODEL OF ACUTE NECROTIZING PANCREATITIS IN RATS: CHEMISTRY, HISTOLOGIC FINDINGS AND SURVIVAL PATTERN.

B. van Ooijen (1), C.J. Tinga (2), W.J. Kort (1), J.H.P. Wilson (3), D.L. Westbroek (1).

Departments of Experimental Surgery (1) and Internal Medicine (3), Erasmus University, Rotterdam; Department of Pathology (2), Bronovo Hospital, The Hague.

5.1. ABSTRACT

Acute necrotizing pancreatitis in rats could be effectively induced by means of retrograde injection of a sodium taurocholate solution (5% w/v) into the biliopancreatic duct. All histological features were consistent with acute pancreatitis, but the extensive development of fat necrosis and the percentage acinar necrosis differed markedly from that seen in a control group, which received sodium chloride (0.9% w/v) instead of sodium taurocholate.

The induction of acute necrotizing pancreatitis by means of sodium taurocholate is associated with a 100% mortality rate within 30 hours. There was no mortality in the control group. The model is simple, easy to perform, relatively cheap and leads to lesions of uniform severity.

5.2. INTRODUCTION

Despite many investigations the pathogenesis and pathophysiology of acute pancreatitis have not yet been clarified. Opie proposed in 1901 the common channel theory to explain the pathogenesis of acute pancreatitis (1). He suggested that reflux of bile into the pancreatic duct system is important for the development of the disease. Other mechanisms have since been proposed including duct destruction, impaired pancreatic mucosal barrier, capillary deterioration or an imbalance between activated proteases and their inhibitors (2). In addition, ischemia and impairment of the pancreatic microcirculation may play a role, possibly in the development and definitely in the course of

the disease (3,4).

A reproducible experimental model is needed to study the pathogenesis of acute pancreatitis. The common methods of inducing experimental acute pancreatitis consist of the injection of trypsin, bile, bile salts, activated pancreatic juice or combinations of these agents into the pancreatic duct (5,6).

The purpose of the present study is to show the reliability of an experimental model in which pancreatitis is easily induced and the progression of pancreatic changes can be studied.

5.3. MATERIALS AND METHODS

Induction of pancreatitis:

For this study 105 inbred male Wag/Rij rats, weighing 200-250 grams, were used. They were allowed to eat and drink ad libitum. There were 65 rats in the experimental group and 40 rats in the control group. All 105 animals underwent laparotomy through a midline incision under light ether anesthesia. A needle was introduced into the biliopancreatic duct via the duodenum, according to the method of Heinkel (7). The bile ducts were clamped at the level of the hilus. The needle was fixed at the entrance to the duodenum with a small bulldog clamp. Sodium taurocholate (5% w/v) was administered at a rate of 0.1 ml/min under careful steady manual pressure to the experimental group in a dose of 0.1 ml/100 g body weight. The control group received 0.9% NaCl w/v instead of sodium taurocholate (STC). The needle and clamps were removed and the midline incision closed in a single layer. The procedure lasted 10 minutes.

Follow up:

Eight animals of the control group and ten animals of the experimental group were sacrificed 3, 6 and 12 hours after the laparotomy. The whole pancreas was removed with duodenum and spleen attached. The pancreas was then freed of the duodenum and spleen, fixed in 4% buffered formalin and embedded in paraffin. Sections of the whole gland were cut and stained with hematoxylin and eosin.

Sixteen animals of the control group and twenty animals of the experimental

group were allowed to live for up to 72 hours. Survival time was recorded and survivors were sacrificed at 72 hours. The pancreas was removed and prepared as described above. Sections were assessed by light microscopy.

Fifteen other animals of the experimental group were used for assessment of amylase and lipase activity. Blood (0.5 ml) was withdrawn just before the laparotomy (zero time) and twelve hours afterwards. Blood samples were drawn from the orbital venous plexus via a heparinized capillary tube. The samples were immediately centrifuged and the serum stored at -30 degrees C until assay. Blood samples were replaced with an equal volume of saline given subcutaneously.

Analytical methods:

Amylase activity was determined using the enzymatic color test of Boehringer Mannheim (Automatenpackung Boehringer Mannheim a-amylase DHP, no. 568 589) with p-nitrophenyl-alpha,D-malto-heptaosid as substrate. The activity of amylase was expressed in units/l.

Lipase activity was determined using the UV-test of Boehringer Mannheim (Automatenpackung Boehringer Mannheim lipase, triacylglycerol lipase E.C. 3.1.1.3. UV test, no. 262 358). This method was described by Ziegenhorn and co-workers (8). The activity of lipase was expressed in units/l.

Histological assessment:

The sections of the pancreas were assessed by a pathologist who was unaware of the treatment group. The following details were assessed:

- inflammatory response; score 0 = scattered, 1 = mild, 2 = moderate, 3 = severe.
- interlobular edema; score 0 = no evidence, 1 = mild, 2 = moderate, 3 = severe.
- hemorrhages; score 0 = small, 1 = large.
- vascular response: infiltration of vascular walls by granulocytes, fibrinoid necrosis of small and large vessels.
- ductal changes: disintegration of ductal walls, dilatation of ducts.
- acinar changes: dilatation of acini; amount of acinar necrosis: score 0 = not present, 1 = <10% of the pancreas, 2 = 10-25%, 3 = 25-50%, 4 = >50%.

Statistical analysis of the amylase, lipase and survival data was carried out by the Mann Whitney U test, statistical analysis of the histological scores by the chi-square test.

5.4. RESULTS

Survival:

All animals in the control group survived for more than 72 hours. All animals in the test group died within 30 hours: 35% died within 12 hours, 59% between 12 and 24 hours and 6% between 24 and 29 hours.

Chemistry:

A significant increase ($p < 0.01$) in the serum levels of amylase and lipase was seen after the induction of acute necrotizing pancreatitis. Serum amylase levels increased up to fourfold of the baseline levels (from 9547 $\pm$ 561 U/l to 35378 $\pm$ 6303 U/l (means $\pm$ s.d.)). Serum lipase levels rose from zero U/l at zero time to 4199 $\pm$ 1375 U/l twelve hours after induction.

Macroscopic findings:

Three hours after the laparotomy no abnormalities were seen in the control group, except for a somewhat edematous pancreas in some cases. In two rats a small amount of serous ascitic fluid had accumulated. In one rat a small hematoma was found in the pancreas. In the experimental group some serosanguineous ascitic fluid was encountered in all rats. The pancreas, clearly swollen in all rats, had a yellowish-brownish color in all but one case. A few spots of fat necrosis were seen on the retroperitoneum, predominantly in the region of the pancreas. In one rat dilatation of duodenum and stomach was seen.

Six hours after the laparotomy the control animals exhibited no clear abnormalities. In the experimental group there was considerable serosanguineous ascitic fluid, while the pancreas was swollen and yellow-brown in color. Large areas of fat necrosis were seen on the retroperitoneum in six

rats, while in four there were even large areas of fat necrosis in the mesentery, greater sac and epididymal fat. In four rats a paralytic ileus of the proximal small bowel had developed.

Twelve hours after the laparotomy there were still no abnormalities to be seen in the control group except for attachment of the liver to the puncture site at the duodenum in a few instances. In the experimental group two animals died within 12 hours. In comparison with the six-hour group the amount of serosanguineous ascitic fluid had increased, while the areas of fat necrosis on the retroperitoneum and in the mesentery, greater sac and epididymal fat had become even more pronounced. The pancreas was brownish in color. A paralytic ileus had developed in most animals. In a few rats a light to moderate hydrothorax was found, accompanied in three by congestion of the lungs. In one rat congestion of the liver was observed.

The control animals that were allowed to live for up to 72 hours exhibited no abnormalities. The test animals, all died within 30 hours, exhibited the same histologic pattern as after twelve hours.

Microscopic findings:

The inflammatory response was usually mild, but intensified with time. Interlobular edema developed in all animals and was maximal at 3 hours in the experimental group and 6 hours in the control group. Small hemorrhages were noted in all groups (table 1).

Infiltration of blood vessels by granulocytes was seen in about 60% of the experimental and control animals within 6 hours. After 12 hours the blood vessels of 57% of the control animals and 80% of the experimental animals showed infiltration by granulocytes. There were no differences in fibrinoid necrosis of small blood vessels between the two groups. Fibrinoid necrosis of the large blood vessels was noted in 11% of the experimental animals that were allowed to live as long as 72 hours. Visible disintegration of ductal walls was noted in only a few of the experimental animals. Ducts as well as acini in assessable areas of the gland were usually dilated after injection of either 0.9% NaCl or 5% STC (table 2).

The amount of acinar necrosis differed significantly among the experimental and control groups (figure 1,2). At 3 hours more than 25% necrosis was already observed in STC-treated animals. The amount increased slightly with time. In

the control group some acinar necrosis was also seen, but it did not involve more than 25% of the gland. It was sometimes difficult to distinguish between intrapancreatic fat necrosis and necrosis of the acini (table 3).

Table 1: INFLAMMATORY RESPONSE, INTERLOBULAR EDEMA AND HEMORRHAGES IN RATS WITH ACUTE PANCREATITIS AND CONTROLS.

group		IR	(p)	E	(p)	H	(p)
control	(t=3)	0.9±0.2		1.8±0.4		0.3±0.2	
			(NS)		(0.05)		(NS)
AP	(t=3)	0.5±0.3		2.5±0.3		0.2±0.2	
control	(t=6)	1.0±0.3		2.4±0.4		0.0±0.0	
			(NS)		(NS)		(NS)
AP	(t=6)	0.9±0.4		2.0±0.3		0.1±0.2	
control	(t=12)	1.0±0.4		1.7±0.2		0.1±0.2	
			(NS)		(NS)		(NS)
AP	(t=12)	1.3±0.2		1.7±0.2		0.2±0.2	
control	(t=free)	1.4±0.3		1.1±0.2		0.1±0.2	
			(NS)		(0.01)		(NS)
AP	(t=free)	1.3±0.3		2.1±0.4		0.1±0.1	

The results are given as the mean ± s.e. of the scores. IR = inflammatory response (scores 0 = scattered, 1 = mild, 2 = moderate, 3 = severe). E = interlobular edema (scores 0 = no evidence, 1 = mild, 2 = moderate, 3 = severe). H = hemorrhages (scores 0 = small, 1 = large). $p < 0.05$, 0.01 statistically significant differences (chi-square test).

Table 2: VASCULAR RESPONSES, DUCTAL CHANGES AND ACINAR CHANGES IN RATS WITH ACUTE PANCREATITIS AND CONTROLS.

	vascular		ductal	acinar
group	VIBP	FNISV	DOD	DOA
control (t=3)	63	25	75	63
AP (t=3)	60	20	90	80
control (t=6)	63	0	75	75
AP (t=6)	67	11	89	88
control (t=12)	57	14	100	86
AP (t=12)	80	30	100	70

VIBP = vascular infiltration by granulocytes; FNISV = fibrinoid necrosis of small vessels; DOD = dilatation of ducts; DOA = dilatation of acini. The results are given as the percentage of the number of sections evaluated per category. No significant differences were seen between the corresponding groups.

Table 3: AMOUNT OF ACINAR NECROSIS

group	score	group	score	p value
control (t=3)	1.3±0.2	AP (t=3)	2.9±0.5	< 0.01
control (t=6)	2.1±0.2	AP (t=6)	3.1±0.2	< 0.001
control (t=12)	1.9±0.7	AP (t=12)	3.1±0.4	< 0.05
control (free)	1.7±0.4	AP (free)	3.1±0.5	< 0.001

The amount of necrosis is expressed as a percentage of the whole gland on the basis of microscopic evaluation of the slides performed by a pathologist who was unaware of the treatment group. Mean scores ± s.e. are given. Scores: 0 = not present, 1 = <10% of the pancreas, 2 = 10-25%, 3 = 25-50%, 4 = >50%. P value: chi-square test for 2x2 tables: <25% versus >25% acinar necrosis.

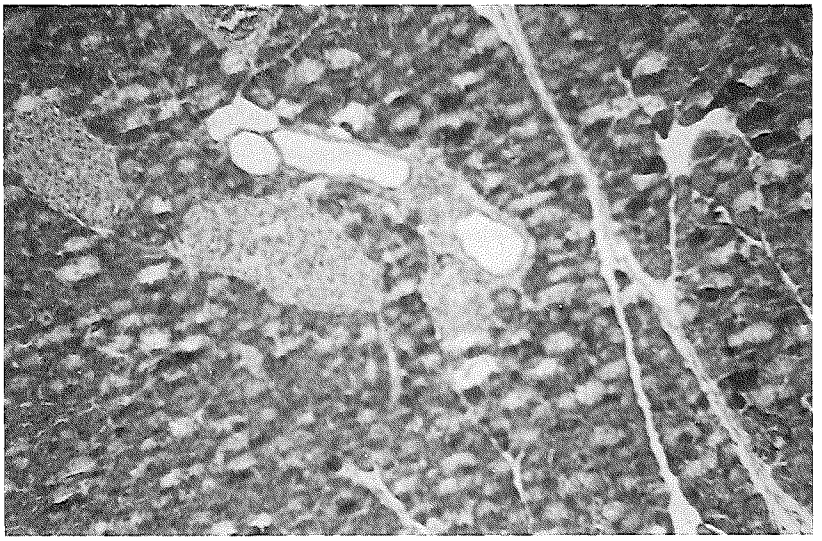

Figure 1: The pancreas 3 hours after injection of 0.9% NaCl (magnification 100x).

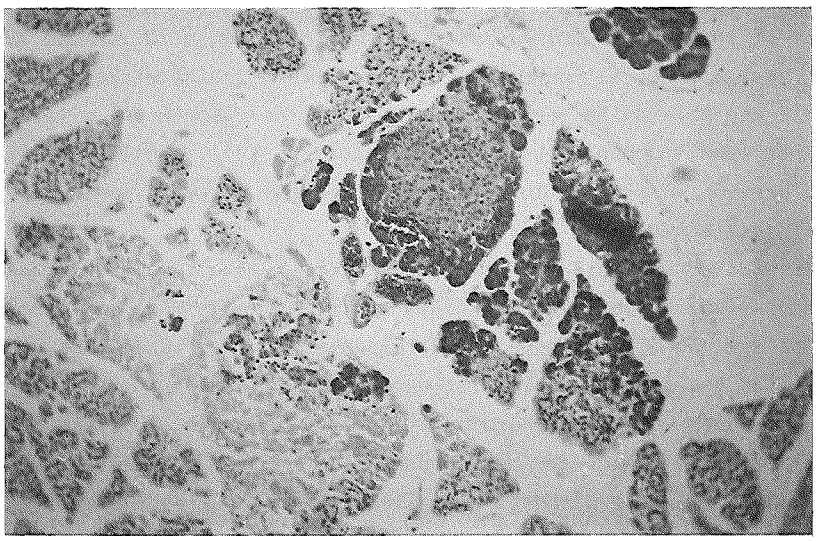

Figure 2: The pancreas 3 hours after injection of 5% STC. Mind the preserved isle of Langerhans (magnification 100x).

5.5. DISCUSSION

The present study demonstrates that injection of a 5% solution of sodium taurocholate, a bile salt, into the biliopancreatic duct of rats induces histological features that are similar to those of clinical acute necrotizing pancreatitis (ANP) in man (9,10). It is a simple model that creates pancreatic lesions of uniform severity. Amylase and lipase levels are markedly elevated. Mortality between 9 and 30 hours is 100%.

Over the years a number of experimental models of acute pancreatitis have been developed, all with their own advantages and disadvantages (5,6). Since the pathogenesis of acute pancreatitis still has not been clarified, no one model can be regarded as far superior to the others. The duct-injection model is in accordance with the theory that acute pancreatitis is caused by a flux of bile and bile salts into the pancreatic duct system. A clinical analogue is gallstone-associated pancreatitis. When a stone obstructs the ampulla or when edema and sphincter spasms occur after passage of a stone, reflux of bile through a common channel induces acute pancreatitis (11,12). This flux of bile is believed to cause ductal hypertension.

Ductal hypertension alone will not induce a lethal form of experimental ANP; none of the control animals died of the injection of 0.9% NaCl into the biliopancreatic duct, although some of the histological features of ANP were seen. Bile and bile salts are necessary for the development of a lethal form of ANP. The constituents of bile may be important (13). A biliodigestive fistula, preventing bile reflux, also prevents mortality in sodium taurocholate-treated rats (14).

Acinar and fat necrosis are common and extensive in this model of ANP. Three hours after induction a few spots of fat necrosis were noted, but at 6 hours necrosis had become marked. The release of lipolytic enzymes is necessary for the development of fat necrosis (15). Acinar necrosis is already marked at 3 hours in sodium taurocholate-treated animals. Interestingly, small areas of acinar necrosis were also observed in control animals, probably due to the rupture of small ductules with local ischemia after the NaCl injection. Significantly more areas of necrosis occurred in the experimental groups; these areas usually involved more than 25% of the pancreas. For example, for the animals that died of the disease within 30 hours the mean score for

necrosis was 3.1. For the control group that was sacrificed after 72 hours, the mean score was 1.7. The extent of necrosis is more than that reported by others (6,14), but also in human acute pancreatitis necrosis may involve 40-70% of the organ (9).

Sodium taurocholate exerts a detergent action on cellular membranes; it dissolves the cellular membrane in ductal walls and destroys adjacent lobules (16,17). A biliodigestive fistula did not prevent the initial acinar necrosis (14). Sodium taurocholate is cleared from the pancreas in less than two hours (6) and if the ampulla remains unobstructed, the progression in the pathological changes after that period is secondary in character. Probably activation of proteolytic enzymes is responsible for the spread of the lesions by autodigestion. Activation of pancreatic enzymes within the gland has been reported during bile or bile salt-induced acute pancreatitis (6,17).

Most of the necrotic changes in the pancreas are thought to be a result of coagulative necrosis (1,9). Ischemia is an important cause of coagulative necrosis which is mediated by phospholipases (18). Ischemia is important in ANP (3,4), and the pancreas becomes ischemic during the course of ANP (6). Opie (1901) and Thal (1957) already considered ischemia to be an important factor in the development of pancreatic lesions (19). They based their view on the fact that acute infiltration of inflammatory cells occurred exclusively in the periphery of hemorrhagic areas.

Phospholipase A2 is a pancreatic enzyme. Bile salts are believed to activate phospholipase A2 (1). Phospholipase A2 converts lecithin to lysolecithin, that is highly cytotoxic (20). The lysolecithin concentration is increased after the sodium taurocholate injection (21). Pancreatic necrosis, activation of phospholipase A2 and ischemia are therefore probably interrelated.

Systemic and intrapancreatic vascular changes are believed to be factors or cofactors in the development of necrosis in human acute pancreatitis (15). Vascular changes occur in ANP (5,19,22). Small hemorrhages were seen in our model after 3 hours. The extravasation of erythrocytes is believed to be caused by disruption of capillaries as well as vascular thrombosis (17,22). Infiltration of polymorphonuclear leukocytes into vascular walls was also seen. Bile salts may have a detergent effect on the vascular endothelium (16). However, no differences in vascular response between the experimental and the control group were seen.

Although the histological features were consistent with ANP, no statistically significant differences in inflammatory response, hemorrhages and ductal and acinar dilatation were seen between control and experimental animals. However, there was a clear-cut difference in the percentage acinar necrosis. Fat necrosis in the peritoneal cavity was also not seen in control animals. These findings have to be kept in mind when the effects of certain therapies are evaluated.

In conclusion, the present study demonstrates that when a 5% sodium taurocholatesolution is injected into the biliopancreatic duct of the Wag Rij rat, reproducible pancreatic lesions develop which are histologically consistent with severe ANP. The rapid development of the lesions was associated with a 100% mortality within 30 hours. The model can be used to study the pathogenesis of acute pancreatitis and also to evaluate methods of treatment. The model is simple, easy to perform and relatively cheap. A potential disadvantage is that care is required to control the pressure when injecting the sodium taurocholate (23), but our histological results support the view that when it is administered under steady manual pressure at a rate of 0.1 ml/min lesions of uniform severity will be induced.

5.6. REFERENCES

1. Creutzfeldt W, Schmidt H. Aetiology and pathogenesis of pancreatitis (current concepts). Scand J Gastroent 1970;6(S):47-62.

2. Lasson A. Acute pancreatitis in man. A clinical and biochemical study of pathophysiology and treatment. Scand J Gastroenterol 1984;99(S):1-57.

3. Popper HC, Neckeles H, Russell KC. Transition of pancreatic edema into pancreatic necrosis. Surg Gyn Obstet 1948;87:79-82.

4. Warshaw AL, O'Hara PJ. Susceptibility of the pancreas to ischemic injury in shock. Ann Surg 1978;188:197-201.

5. Schiller WR, Suriyapa C, Anderson MC. Current research review. A review of experimental pancreatitis. J Surg Res 1974;16:69-90.

6. Aho HJ, Nevalainen TJ, Aho AJ. Experimental pancreatitis in the rat. Development of pancreatic necrosis, ischemia and edema after intraductal sodium taurocholate injection. Eur Surg Res 1983;15:28-36.

7. Heinkel K. Die ratte als versuchtier in der experimentelen pankreasdiagnostik. II Mitteilung: die erzeugung einer akuten hamorrhagischen pankreatitis durch injektion von gallensaure in den duktus pankreatikus. Klin Wochenschr 1953;31:815.

8. Ziegenhorn J, Neumann KW, Zwez W. Determination of serum lipase. Clin Chem 1979;25:1067.

9. Phat VN, Guerrieri MT, Alexandre JH, Camilleri JP. Early histological changes in acute necrotizing hemorrhagic pancreatitis. A retrospective pathological study of 20 total pancreatectomy specimens. Path Res Pract 1984;178:273-279.

10. Foulis AK. Histological evidence of initiating factors in acute necrotizing pancreatitis in man. J Clin Path 1980;33:1125-1131.

11. Kelly TR. Gallstone pancreatitis. Arch Surg 1974;109:294-297.

12. Acosta JM, Ledesma CL. Gallstone migration as a cause of acute pancreatitis. N Engl J Med 1974;290:484-487.

13. Armstrong CP, Taylor TV, Torrance HB. Lecithin is important in maintaining pancreatic duct integrity. Gut 1984;25:1174.

14. Lange JF, Van Gool J, Tytgat GNJ. Bile reflux in experimental acute pancreatitis in the rat. Eur Surg Res 1986;18:369-375.

15. Nordback I, Lauslahti K. Clinical pathology of acute necrotising pancreatitis. J Clin Pathol 1986;39:68-74.

16. Aho HJ, Koskensalo SM-L, Nevalainen TJ. Experimental pancreatitis in the rat. Sodium taurocholate-induced acute haemorrhagic pancreatitis. Scand J Gastroent 1980;15:411-416.

17. Aho HJ, Nevalainen TJ. Experimental pancreatitis in the rat. Ultrastructure of sodium taurocholate-induced pancreatic lesions. Scand J Gastroent 1980;15:417-424.

18. Farber JL. Biology of disease. Membrane injury and calcium homeostasis in the pathogenesis of coagulative necrosis. Lab Invest 1982;47:114-23.

19. Rao SS, Watt IA, Donaldson LA, Crocket A, Joffe SN. A serial histologic study of the development and progression of acute pancreatitis in the rat. Am J Pathol 1982;103:39-46.

20. Aho HJ, Nevalainen TJ. Experimental pancreatitis in the rat. Light and electron microscopical observations on early pancreatic lesions induced by intraductal injection of trypsin, phospholipase A2, lysolecithin and non-ionic detergent. Virchows Arch (Cell Pathol) 1982;40:347-356.

21. Aho HJ, Nevalainen TJ, Lindberg RLP, Aho AJ. Experimental pancreatitis in the rat. The role of phospholipase A in sodium taurocholate-induced acute haemorrhagic pancreatitis. Scand J Gastroent 1980;15:1027-1031.

22. Nuutinen P, Kivisaari L, Standertskjold-Nordenstam C-G, Lempinen M, Schroder T. Microangiography of the pancreas in experimental oedemic and haemorrhagic pancreatitis. Scand J Gastroenterol 1986;21(S26):12-17.
23. Steer ML. Workshop on experimental pancreatitis. Dig Dis Sci 1985;30:575-581.

CHAPTER 6

RAISED PLASMA THROMBOXANE B2 LEVELS IN EXPERIMENTAL ACUTE NECROTIZING PANCREATITIS IN RATS. THE EFFECTS OF FLUNARIZINE, DAZOXIBEN AND INDOMETHACIN *.

Bart van Ooijen (1), Rob J.T. Ouwendijk (2), Wil J. Kort (1), Freek J. Zijlstra (3), J.Eric Vincent (3) J.H.Paul Wilson (2), Dick L. Westbroek (1).

Departments of Experimental Surgery (1), Internal Medicine (2) and Pharmacology (3), Faculty of Medicine, Erasmus University, Rotterdam.

6.1. ABSTRACT

The possible role of thromboxane A2 (TXA2) in acute necrotizing pancreatitis (ANP) was investigated in rats. After ANP was induced by injecting sodium taurocholate (5% w/v) into the pancreatic duct, the thromboxane B2 (TXB2) levels in plasma increased significantly. The effects of indomethacin, a general blocker of prostaglandin synthesis, on survival time and on plasma TXB2 levels were compared with those of dazoxiben, a more specific blocker of TXA2 synthesis, and flunarizine, a calcium entry blocker that is known to inhibit the effects of TXA2. In a test group without any treatment, all animals died within 30 hours of the induction of ANP. Although TXB2 levels were lowered by the administration of indomethacin, dazoxiben and flunarizine, survival times were not significantly altered. Indomethacin pretreatment had no beneficial effect, while 30% and 40% of the animals survived for 36 hours after treatment with flunarizine and dazoxiben, respectively. The results of the present study indicate that inhibition of TXA2 synthesis alone does not dramatically alter survival time. However, a potential role for other arachidonate metabolites in ANP cannot be ruled out by this study.

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6.2. INTRODUCTION

As long ago as 1896 Chiari suggested that the main cause of pancreatitis might be autodigestion of the pancreas by pancreatic enzymes. Trypsin, originally thought to be the main culprit, does not seem to play an essential role since several studies on the effects of aprotinin (Trasylol (R)) have reported disappointing results. Nevalainen et al. (1) have shown by immunohistochemical techniques that the phospholipase A2 (PLA2) content of pancreatic tissue is elevated in patients with acute pancreatitis. In addition raised serum levels of PLA2 have been found (2). PLA2 splits off a single fatty acid from the 2-position of membrane phospholipids. In this reaction lysophospholipids and mainly polyunsaturated fatty acids are formed.

The polyunsaturated fatty acids include arachidonic acid, the major precursor of prostaglandins and leukotrienes. Thromboxane A2 (TXA2), a product of prostaglandin synthesis, causes marked vasoconstriction and platelet aggregation (3) and also plays a role in shock (4). Therefore, TXA2 could be responsible for the decreased perfusion of the pancreas that occurs in acute pancreatitis (5-8), leading to ischemia and cell death. Ischemia has been reported to play an important role in acute pancreatitis (5,9,10). In addition, TXA2 could be a mediator of the complications of acute pancreatitis, such as renal insufficiency, pulmonary insufficiency and hepatic failure.

In view of the importance of ischemia and shock in acute pancreatitis we performed a study to measure TXA2 formation. We tested the effects of indomethacin, a general blocker of prostaglandin synthesis, dazoxiben, a specific blocker of thromboxane A2 synthesis (11), and flunarizine (R), a calcium entry blocker. Flunarizine inhibits the action of vasoconstrictors such as TXA2 (12). The effects of these agents on survival time and the plasma levels of TXB2 in rats are presented here.

6.3. MATERIALS AND METHODS

Acute necrotizing pancreatitis (ANP) was induced in inbred male Wag/Rij rats, weighing 200-250 grams. After a midline laparotomy under light ether anesthesia, a needle was introduced into the biliopancreatic duct via the duodenum, according to the method of Heinkel (13). The hepatic ducts were clamped at the level of the hilus and 5% sodium taurocholate (STC) (0.1 ml/100

g body weight) was injected under steady manual pressure at a rate of 0.1 ml/min. A control group received 0.9% sodium chloride instead of STC.

Dilutions of indomethacin (Merck, Sharp and Dohme, Haarlem, The Netherlands)(0.2 mg/ml) were prepared on the day of injection. Test animals received a dose of 1 mg/kg indomethacin intravenously just prior to the laparotomy . Dazoxiben was obtained as a gift from Pfizer Central Research Laboratories, Sandwich, England. Dilutions (5 mg/ml) were prepared on the day of injection. Test animals received a dose of 30 mg/kg dazoxiben intravenously just prior to the laparotomy. Flunarizine (R) was a gift of Janssens Pharmaceuticals, Goirle, The Netherlands. Dilutions in physiological saline (0.1 mg/ml) were prepared on the day of injection. Test animals were given flunarizine intravenously just before the laparotomy in a dose of 0.1 mg/kg.

The rats were randomly assigned to five groups:

group A (n= 8) Control group : no therapy.
group B (n=10) Experimental AP group: no therapy.
group C (n=10) Experimental AP group: flunarizine therapy.
group D (n=10) Experimental AP group: dazoxiben therapy.
group E (n=10) Experimental AP group: indomethacin therapy.

Blood was drawn for TXB2 determinations before the laparotomy and drug administration (zero time) and three, six and twelve hours after the induction of ANP. Blood (0.5 ml) was drawn from the orbital venous plexus via a heparinized capillary tube and collected in tubes containing 20 mu-liter of heparin (Thromboliquine (R) 5000 U/ml) and 50 mu-liter indomethacin (0.1 mg/ml in 0.1 M phosphate buffer, pH 8). The blood samples were centrifuged immediately at 1400 x g for 10 minutes; plasma was stored at -70 degrees C until assay.

Plasma TXB2 levels were determined in duplicate by radioimmunoassay as described earlier (14) using charcoal-treated control samples for each experimental sample because of the non-specific binding of plasma proteins.

Anti-thromboxane B2 for the immunoassay was obtained from l'Institute

Pasteur, 3H-TXB2 from Amersham and TXB2 from Sigma.

The survival times were recorded while survivors were sacrificed 72 hours after the laparotomy. The pancreas was removed, fixed in 4% buffered formalin and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin. The sections were examined by light microscopy and the percentage necrosis was assessed by an independent pathologist.

The results for the various groups were compared by the Mann Whitney U test.

6.4. RESULTS

Acute pancreatitis was effectively induced by means of the taurocholate injection into the biliopancreatic duct in all cases, as proven by histological examination. Light microscopic examination of the pancreas showed an inflammatory infiltration with large areas of necrosis. The inflammatory infiltrate was not pronounced in the first 24 hours, the period within which most of the untreated animals (group B) died. The longer the animals survived the more the inflammatory reaction intensified, exemplifying the acute character of the disease. Hydrothorax developed in some of the animals that died in the first 24 hours. In some cases a distended stomach with signs of a paralytic ileus of the proximal bowel was seen. Fat necrosis was pronounced 24 hours after induction and tended to increase with time.

Ascites developed in all animals that died. The largest amounts of ascitic fluid were found in the first 36 hours. A slight inflammatory infiltrate was the only finding for the control group (group A), sometimes with small areas of acinar cell necrosis. Flunarizine-pretreated animals exhibited less necrosis of the pancreas. The percentage necrosis found is summarized in table 1.

All animals in the control group survived for 72 hours. All animals in the non-pretreated pancreatitis group and 9 of the 10 treated with indomethacin died within 30 hours. Of the 10 animals treated with flunarizine 3 survived for more than 36 hours, while 4 of the 10 receiving dazoxiben survived. One rat of both the flunarizine and dazoxiben group died after 36 hours. Survival rates are given in table 2. The differences were not statistically significant.

Table 1. Percentage necrosis in different groups with acute necrotizing pancreatitis.

	amount of necrosis in % *			
group	<10	10-25	25-50	50-100
A:controls	8	-	-	-
B:pancreatitis	-	1	4	4
C:AP + flunarizine	2	2	5	-
D:AP + dazoxiben	2	2	2	3
E:AP + indomethacin	-	1	4	5

*Necrosis, expressed as a percentage of the whole gland, is based on a double blind microscopical evaluation of 45 slides. The number of slides is indicated.

Table 2. Mortality rate for acute necrotizing pancreatitis in rats.

	time period		
group	0-36	36-72	0-72
A:controls	0	0	0
B:pancreatitis	100	0	100
C:AP+flunarizine	70	10	80
D:AP+dazoxiben	60	10	70
E:AP+indomethacin	90	0	90

The mortality rate is expressed as the percentage animals that died in the given time periods. Differences were not significant.

The plasma TXB2 levels rose significantly after induction of acute necrotizing pancreatitis compared to those found for control group A ($p<0.05$ and $p<0.01$). After the injection of 0.9% sodium chloride only a slight increase in TXB2 levels was seen. Twelve hours after induction of ANP the TXB2 levels were declining, although the difference between experimental group B and the control group A was still significant. TXA2 synthesis was effectively inhibited by administration of both indomethacin and dazoxiben, as reflected by the pronounced decrease in TXB2 levels. Inhibition was less pronounced after flunarizine-pretreatment. The mean values of the TXB2 concentrations at various points in time are shown in table 3. In table 4 the TXB2 values are expressed as a percentage of the level at zero time. The maximum per cent change in TXB2 levels was reached six hours after induction of ANP. TXB2 levels dropped below the initial levels in group D (dazoxiben) and E (indomethacin). The higher level at zero time must be ascribed to additional factors, such as ether anesthesia, etc. Administration of the antagonists effectively inhibited all subsequent production of TXB2.

Table 3. Mean TXB2 levels found for the different groups.

	time in hours			
group	t=0	t=3	t=6	t=12
A:control	149±35	172± 30	203± 47	181± 48
		*	+	+
B:AP	153± 8	423±163	626±368	410±116
C:AP+F	171±20	240± 34	279± 47	307± 45
D:AP+D	119±16	<100	<100	<100
E:AP+I	134±20	131± 25	163± 53	137± 48

The plasma values are given in pg/ml as the mean ± SD.
* $p<0.05$ significant with respect to the control group
+ $p<0.01$ significant with respect to the control group
F=flunarizine, D=dazoxiben, I=indomethacin.

Table 4. Changing levels of plasma thromboxane B2.

	time in hours			
group	t=0	t=3	t=6	t=12
A:control	100	115	136	121
		*	+	+
B:pancreatitis	100	276	409	268
C:AP+flunarizine	100	140	163	179
D:AP+dazoxiben	100	84	84	84
E:AP+indomethacin	100	98	122	102

The values are expressed as a percentage of the mean baseline values.
* $p < 0.05$ significant with respect to the controls.
+ $p < 0.01$ significant with respect to the controls.

6.5. DISCUSSION

The exact cause of acute pancreatitis remains uncertain. Although many aspects of this disease have been investigated the pathogenesis is still a point of discussion. Several mechanisms have been suggested including destroyed ducts, reflux of duodenal contents, an impaired pancreatic mucosal barrier, damaged capillaries or an imbalance between activated proteases and their inhibitors (15). In addition, ischemia and disturbances of the pancreatic microcirculation seem to play an important role in either the initiation or the course of the disease (5,9). Ischemia is probably a critical factor in the progression of pancreatic edema to pancreatic necrosis (10). The inflamed pancreas is even more vulnerable to ischemia. Therefore, mediators which cause disturbances in the pancreatic microcirculation need further study. Thromboxane A2 (TXA2) is such a mediator. TXA2 causes marked

vasoconstriction and platelet aggregation (3). We measured TXB2 levels (the stable metabolite of TXA2) in plasma after induction of ANP and found a marked elevation three and six hours after induction (table 3). Highly significant percentual changes ($p < 0.01$) could be demonstrated (table 4).

However, blockage of TXA2 synthesis by pretreatment with indomethacin did not improve survival since 90% of the animals still died within 30 hours. Since indomethacin is a general blocker of prostaglandin synthesis, it is conceivable that other prostaglandins that may protect the pancreatic circulation and the pancreatic cell are eliminated by this drug. Pretreatment with dazoxiben, a more specific blocker of TXA2 synthesis (11), decreased the mortality rate by 40%. Dazoxiben is said to cause a slight redirection of TXA2 towards prostacyclin (PGI2) (16). Thus, the increase in survival rate may also be caused by increased PGI2 synthesis instead of blockage of TXA2.

Pretreatment with flunarizine, a calcium entry blocker, resulted in inhibition of the TXB2 increase, but no significant effect on survival was observed since 30% of the animals survived. Interestingly, no severe necrosis was seen in the histologic specimens of flunarizine-treated animals (table 1). It is possible that flunarizine interacts with phospholipase A2. PLA2 is thought to play a major role in pancreatic necrosis in ANP (17) and inhibition of PLA2 is indeed associated with diminished necrosis (18). Flunarizine acts as a TXA2 antagonist (12). The decrease in endogenous TXB2 levels caused by flunarizine may possibly be attributable to inhibition of the release of arachidonic acid by PLA2. Phospholipase A2 is strongly dependent on calcium (19). The pancreatic necrosis in acute pancreatitis has been attributed to accelerated degradation of membrane phospholipids (20). This degradation results in membrane deterioration and thus an altered permeability for calcium ions. Influx of calcium ions will lead to cell death and cell necrosis (20). It is the disturbance of membrane function that characterizes the loss of reversibility of ischemic injury. Whether flunarizine works in this way in pancreatic necrosis is not yet clear.

In summary our results show that TXB2 levels are elevated in ANP. However, inhibition of TXA2 synthesis does not alter survival significantly, which makes an exclusive role for TXA2 unlikely. The 40% decrease in mortality by pretreatment with dazoxiben justifies further studies to examine the possible effects of other prostaglandins in ANP.

6.6. REFERENCES

1. Nevalainen TJ. Immunohistochemical localisation of phospholipase A2 in human pancreas in acute and chronic pancreatitis. Acta Pathol Microbiol Immunol Scand 1983;91(A):97-102.
2. Schroder T, Kivilaakso R, Kinnunen PKJ, et al. Serum phospholipase A2 in human acute pancreatitis. Scand J Gastroent 1980;15:633-636.
3. Robert A. Prostaglandins and digestive diseases. Adv Prostaglandin Thromboxane Res 1980;8:1533-1541.
4. Webb PJ, Westwick J, Scully MF, et al. Do prostacyclin and thromboxane play a role in endotoxic shock? Br J Surg 1981;68:720-724.
5. Goodhead B. Acute pancreatitis and pancreatic bloodflow. Surg Gyn Obstet 1969;69:331-340.
6. Donaldson LA. Pancreatic circulation reduces rapidly in acute pancreatitis. Surgery 1978;84:313-321.
7. Papp M, Makara GB, Hajtman B, et al. A quantitative study of pancreatic blood flow in experimental pancreatitis. Gastroenterology 1966;51:524-528.
8. Schiller WR, Anderson MC. Microcirculation of the normal and inflamed canine pancreas. Ann Surg 1975;181:466-470.
9. Warshaw AL, O'Hara PJ. Susceptibility of the pancreas to ischemic injury in shock. Ann Surg 1978;188:197-201.
10. Popper HC, Neckeles H, Russell KC. Transition of pancreatic edema into pancreatic necrosis. Surg Gyn Obstet 1948;87:79-82.
11. Tyler HM. Dazoxiben: pharmacological tool or clinical candidate? Br J Clin Pharmacol 1983;15(S):13-16.
12. De Clerck F, Van Nueten JM. Platelet-mediated vascular contraction. Inhibition by flunarizine, a calcium entry blocker. Bioch Pharmacol 1983;32:765-771.
13. Heinkel K. Die ratte als versuchtier in der experimentelen pankreasdiagnostik. II Mitteilung: die erzeugung einer akuten hamorrhagischen pankreatitis durch injektion von gallensaure in den duktus pankreatikus. Klin Wochenschr 1953;31:815.
14. Ouwendijk RJT, Zijlstra FJ, Wilson JHP, et al. Raised plasma thromboxane B2 levels in alcoholic liver disease. Prostaglandins Leukotrienes Medicine 1983;10:115-122.
15. Lasson A. Acute pancreatitis in man. Scand J Gastroenterol 1984;99(S):1-57.
16. O'Keefe EH, Liu ECK, Greenberg R, Ogletree ML. Effects of a thromboxane synthetase inhibitor and a thromboxane antagonist on release and activity of TXA2 and prostacyclin in vitro. Prostaglandins 1985;29:785-797.
17. Aho HJ, Nevalainen TJ. Experimental pancreatitis in the rat. Virchows Arch (Cell Path) 1982;40:347-356.
18. Schroder T, Lempinen M, Nordling S, et al. Chlorpromazine treatment of experimental acute fulminant pancreatitis in pigs. Eur Surg Res 1981;13:143-151.
19. Scherphof G, Westeneng H. Stimulation and inhibition of pancreatic phospholipase A2 by local anesthetics as a result of their interaction with the substrate. Biochem Biophys Acta 1975;398:442-452.
20. Farber JL. Biology of disease. Membrane injury and calcium homeostasis in the pathogenesis of coagulative necrosis. Lab Invest 1982;47:114-123.

CHAPTER 7

PROSTANOID IMBALANCE IN EXPERIMENTAL ACUTE NECROTIZING PANCREATITIS IN RATS *.

Bart van Ooijen (1), Wil J Kort (1), Freek J Zijlstra (2),
J Eric Vincent (2), JH Paul Wilson (3), Dick L Westbroek (1).

Departments of Experimental Surgery (1), Pharmacology
(2) and Internal Medicine (3), Faculty of Medicine,
Erasmus University, Rotterdam.

7.1. ABSTRACT

In an investigation of the pathogenesis of acute necrotizing pancreatitis (ANP) the plasma levels of TXB2, 6-keto-PGF1alpha and PGE2 were measured in rats. After induction of ANP by injection of 5% sodium taurocholate into the pancreatic duct, a marked increase in TXB2 levels and a slight increase in 6-keto-PGF1alpha levels were found. PGE2 levels decreased. Mortality was 100 % within 30 hours. Pretreatment with chloroquine (R), a phospholipase A2 inhibitor, led to an inhibition of TXB2 production, while 6-keto-PGF1alpha and PGE2 levels showed a surprising slight elevation in the first six hours. Pretreatment with chloroquine (R) decreased mortality by 30%. Pretreatment with FPL 55712, a leukotriene synthesis blocker, caused an increase in TXB2 and PGE2 levels, while the formation of 6-keto-PGF1alpha remained unaltered. Two out of nine animals survived after pretreatment with FPL 55712. The results of the present study indicate that arachidonate end products are involved in ANP. The significance of the high TXB2 levels, decreased PGE2 levels and only slightly elevated 6-keto-PGF1alpha levels during ANP requires further investigation. The thromboxane A2/prostacyclin ratio may be important.

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7.2. INTRODUCTION

The exact cause of acute pancreatitis is unknown. It is clear from several studies that arachidonate metabolites are involved in the pathophysiology of many diseases (1,2). Recently, we reported elevated thromboxane B2 levels (the stable metabolite of thromboxane A2) in experimental acute necrotizing pancreatitis (ANP) in rats (3). Thromboxane A2 (TXA2) is a strong vasoconstrictor and a potent stimulus of platelet aggregation (1,4). TXA2 may have a detrimental effect on pancreatic blood flow and microcirculation.

Prostacyclin (PGI2) is a potent vasodilator produced in the vascular endothelium and gastrointestinal tract; it is also a powerful inhibitor of platelet and white cell activation (1,5,6). Moreover, the TXA2/PGI2 ratio is reported to be important in vascular homeostasis. The ratio may function as a balance control mechanism for several cellular functions (4,5,7).

Prostaglandin E2 (PGE2) has been shown to play a role in ANP too. It normalizes membrane permeability (8), which may be important in ANP, and influences the blood flow in several ways as well. However, contradictory reports have been published on its beneficial effects on survival in pancreatitis (9,10,11).

The aim of the present study was to investigate in more detail whether prostaglandin production is altered in ANP and whether there are any pathophysiological implications of changes in the TXA2/PGI2 ratio. We measured TXB2, PGE2 and 6-keto-PGF1alpha (the stable metabolite of PGI2) levels in the plasma of rats after induction of ANP and in control rats. We also tested the effects of pretreatment with either chloroquine (R) or FPL 55712 on both eicosanoid synthesis and survival. Chloroquine (R) is a phospholipase A2 (PLA2) inhibitor (12). The supposed action of PLA2 in ANP (12) leads to the liberation of arachidonic acid from phospholipids. Cyclooxygenases metabolize arachidonic acid to prostaglandins and thromboxanes (13) and lipoxygenases to leukotrienes (14). PLA2 blockers influence the course of ANP beneficially (15,16), possibly by inhibiting the metabolism of arachidonic acid. Quantitative measurements of eicosanoid production are not available, however. FPL 55712 is an inhibitor of the 5-lipoxygenase pathway (17). During the course of ANP leukotrienes may be formed in addition to the prostaglandins and the thromboxanes; leukotrienes are responsible for many of the

pathophysiological events in several diseases (18,19). We hoped to determine whether chloroquine (R) and FPL 55712 alter survival in ANP and whether the observed effects can be ascribed to inhibition of the formation of the thromboxanes, leukotrienes or prostaglandins.

7.3. MATERIALS AND METHODS

Inbred male Wag/Rij rats weighing 200-250 grams were used. Acute necrotizing pancreatitis (ANP) was induced by retrograde injection of 5% sodium taurocholate (STC) (0.1 ml/100 g body weight) into the pancreatic duct, as previously reported (3). A control group received 0.9% sodium chloride instead of STC.

Experiment 1: Collection of blood and determination of plasma levels of TXB2, 6-keto-PGF1alpha and PGE2.

Blood (1.0 ml) was collected from the aorta before the induction of ANP (baseline values) and three, six and twelve hours afterwards. The abdominal aorta was exposed after a midline laparotomy under ether anesthesia and then punctured with a 0.5 mm needle. One ml of blood was collected in tubes containing 20 mu-liter of heparin (5000 U/ml Thromboliquine (R)) and 50 mu-liter of indomethacin (0.1 mg/ml in 0.1 M phosphate buffer, pH 8); saline was added to obtain a volume of 1.5 ml. The blood samples were centrifuged immediately at 1400 x g for ten minutes; plasma was stored at -70 degrees C until the assay. All rats were killed after blood was collected. In all groups each value (three, six and twelve hours) was the mean for seven different rats; the baseline value also was the mean for seven rats. Three minutes before blood was drawn, the rats received an intravenous injection of 1 ml heparin (Thromboliquine (R), 50 U/ml) per 150 gram body weight.

The following groups were studied:

group A: control group
group B: experimental ANP group
group C: experimental ANP group pretreated with chloroquine (R) (100 mg/kg)
group D: experimental ANP group pretreated with FPL 55712 (10 mg/kg)

Chloroquine (R) (100 mg/kg) was given orally 24 hours prior to the induction of ANP and again just before induction. A fresh solution was made each day with physiological saline.

FPL 55712 (sodium salt batch 13, a generous gift from Fisons Pharmaceuticals, Leusden, Holland) was administered intravenously just before the induction of ANP and three, six and nine hours after the induction of ANP. FPL 55712 was dissolved in methanol and diluted with 0.9% sodium chloride. One ml of injection solution contained 4 mg FPL 55712, 0.114 ml methanol and 0.886 ml 0.9% sodium chloride.

Plasma TXB2 levels were determined in duplicate by radioimmunoassay, as described previously (20). Plasma PGE2 and 6-keto-PGF1alpha levels were determined in duplicate by specific radioimmunoassays. Because of the non-specific binding of plasma proteins the values after Sep Pak prepurification were below zero. Therefore each experimental sample was assessed in relation to its own control sample. A control sample was obtained by pretreatment of a volume equal to that of the test sample with a charcoal suspension (1:1). Statistical evaluation was performed with the Mann Whitney U test.

Experiment 2: Survival studies.

The survival time for rats treated with chloroquine (R) or FPL 55712 was compared with that found for control groups.

The following groups were studied:

group 1: control group (n=8)
group 2: experimental ANP group (n=10)
group 3: experimental ANP group pretreated with chloroquine (100 mg/kg) (n=10)
group 4: experimental ANP group pretreated with FPL 55712 (10 mg/kg) (n=9)
group 5: control group pretreated with chloroquine (100 mg/kg) (n=5)

Survival times were recorded; animals surviving the 72-hour observation period were sacrificed. The pancreas was removed, fixed in 4% buffered

formalin and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin. The preparations were assessed by light microscopy.

The results for the different groups were compared using Fischer's test for 2x2 tables.

7.4. RESULTS

PART 1: Levels of TXB2, PGE2 and 6-keto-PGF1alpha.

After the induction of ANP (group B) the TXB2 and 6-keto-PGF1alpha values were significantly higher at three and six hours than those found for control group A, which did not exhibit any significant changes in the TXB2, PGE2 or 6-keto-PGF1alpha plasma levels compared to the baseline values at zero time. The elevated TXB2 levels subsequently decreased to slightly elevated levels twelve hours after induction of ANP. The 6-keto-PGF1alpha levels returned to baseline values twelve hours after induction of ANP. PGE2 plasma levels decreased at three and six hours, and were still low twelve hours after the induction of ANP, at which time the difference with group A (controls) was significant.

Chloroquine (R) pretreatment (group C) abolished the rise in TXB2 levels caused by ANP; as a result the difference in TXB2 levels at three hours between group B and group C was significant. The PGE2 levels for group C were slightly elevated at three hours, equal to the baseline values at six hours and low at twelve hours. However, the plasma levels of PGE2 found for group C three and six hours after induction of ANP were significantly higher than those for group B. The elevated levels of 6-keto-PGF1alpha three and six hours after the induction of ANP were also surprising. The levels returned to baseline values at twelve hours.

FPL 55712 pretreatment did not influence the significant rise in TXB2 levels after induction of ANP. However, it caused significant elevation of the PGE2 levels compared to the levels found for both group A (controls) and the pancreatitis group (B). The levels of 6-keto-PGF1alpha remained at baseline value.

The values, expressed as the percentage change in the baseline level (i.e. the level at zero time before induction of ANP), are listed in tables 1, 2 and 3.

Table 1: The change in plasma TXB2 levels after induction of acute necrotizing pancreatitis.

		time period		
group	baseline	t=3	t=6	t=12
A:controls	100	107	60	114
B:AP	100	257 *	310 *	136
C:AP with chloroquine	100	119	107	60
D:AP with FPL 55712	100	236 *	233 *	220

The values are expressed as a percentage of the median baseline value (100%), which equaled 42 pg/ml (range <25-128 pg/ml).

* $p < 0.05$ significant with respect to the control group.

Table 2: The change in plasma 6-keto-PGF1alpha levels after induction of acute necrotizing pancreatitis.

		time period		
group	baseline	t=3	t=6	t=12
A:controls	100	91	76	78
B:AP	100	163 *	168 +	109 *
C:AP with chloroquine	100	150 *	166 +	99
D:AP with FPL 55712	100	118	105	103

The values are expressed as a percentage of the median baseline value (100%), which equaled 845 pg/ml (range 460-885 pg/ml).

* $p < 0.05$ significant with respect to the control group.

+ $p < 0.01$ significant with respect to the control group.

Table 3: The change in plasma PGE2 levels after induction of acute necrotizing pancreatitis.

	time period			
group	baseline	t=3	t=6	t=12
A:controls	100	76	83	120
B:AP	100	71	47	36 *
C:AP with chloroquine	100	143	100	50
D:AP with FPL 55712	100	346 *	270	226

The values are expressed as a percentage of the median baseline value (100%), which equaled 70 pg/ml (range ≤25-178 pg/ml).

* $p < 0.05$ significant with respect to the control group.

PART 2: Survival studies.

All animals of the control group (1) survived the 72-hour observation period. Conversely, all animals of the ANP group (2) died within 30 hours (range 10.5-29 hours) with a mean survival time of 14.8 ± 5.9 hours. Chloroquine pretreatment decreased mortality from 100 % to 70%, which was not statistically significant. None of the five control animals in group 5 died. Of the nine FPL 55712-pretreated animals two survived for more than 72 hours, a difference which also was not statistically significant. A summary of these results is given in table 4.

The induction of ANP by means of retrograde injection of sodium taurocholate into the biliopancreatic duct caused a histological pattern of necrotizing pancreatitis. Light microscopy studies of the pancreas showed an inflammatory infiltrate with large areas of necrosis. Inflammatory infiltration was not pronounced in the first 24 hours, but became severe at 72 hours; apparently, necrosis continued since after 72 hours sometimes more than 80% of the pancreas was necrotic. In the animals who survived for 72 hours a large infiltrate around the pancreas was observed. No differences in histology

between the various ANP groups could be demonstrated.

Ascites occurred in all animals that died within 72 hours. The largest amount of ascitic fluid was found in the first 24 hours, while only small quantities remained in animals, that survived for 72 hours. Hydrothorax developed in some animals that died in the first 24 hours. In several cases a distended stomach with signs of a paralytic ileus of the proximal bowel was found.

Table 4: Survival rate after induction of acute necrotizing pancreatitis in rats.

group	% survival at 72 hours
1:controls (n=8)	100%
2:AP (n=10)	0%
3:AP + chloroquine (n=10)	30% *
4:AP + FPL 55712 (n=9)	22% *
5:controls + chloroquine (n=5)	100%

* No statistically significant difference in mortality

7.5. DISCUSSION

Despite intensive investigation the pathogenetic and pathophysiological events in acute pancreatitis are poorly understood. Ischemia and impairment of the pancreatic microcirculation are presumed to be important (21,22). Since some prostaglandins exhibit potent biological activity, especially in blood vessels (1,5), arachidonate metabolites may play a role in ANP. However, previous studies on the role of some prostaglandins in ANP have so far yielded contradictory results (9,10).

Thromboxane A2 (TXA2) constricts large blood vessels, has a vasoconstrictor effect on the microcirculation and is a potent stimulus of platelet aggregation (1,4). Conversely, prostacyclin (PGI2) is a vasodilator as well as being the most powerful naturally occurring inhibitor of platelet aggregation

yet discovered (4,5,23). The main source of PGI2 is the vessel wall, in particular the intima (5,6). Recently, we reported elevated TXB2 levels (the stable end product of TXA2) in ANP (3). We could reproduce this finding in the present series. Plasma levels of TXB2 rose significantly to three times the baseline values six hours after induction of ANP. We also found elevated plasma 6-keto-PGF1alpha levels (the stable end product of PGI2) in ANP.

Ischemia is said to be associated with elevated TXB2 levels, but not with a rise in 6-keto-PGF1alpha levels (7,24). Some authors have reported that in endotoxic shock (which has frequently been suggested as an important factor in ANP) the concentration of TXB2 was increased (25-27) whereas that of 6-keto-PGF1alpha decreased (25,27,28), while others found an elevated level for both substances (29,30). Moreover, Carmona et al. (27), who induced sepsis in rabbits, found a rise in 6-keto-PGF1alpha and TXB2 levels, while sepsis in a modified model caused depressed levels of 6-keto-PGF1alpha and increased levels of TXB2. Thus differences in experimental design may (at least partially) explain the discrepancy between results.

We found slightly elevated levels (up to 68% of the baseline values) of 6-keto-PGF1alpha in acute pancreatitis. In comparison with group A (control animals), the levels were significantly ($p < 0.05$) increased three and six hours after induction of ANP, but had returned to the baseline values at twelve hours.

Several investigators stress the importance of the TXA2/PGI2 ratio (4,5,27). The TXA2/PGI2 system may be of broad biological significance to cell regulation (4). TXA2 and PGI2 have opposing effects on the cAMP-concentration in cells, thereby providing a balance control mechanism which will affect thrombus and hemostatic plug formation. The precise balance is a major determinant of vascular tone, platelet function and many other cellular homeostatic functions (4). As such the TXA2/PGI2 ratio may also be important for the pancreatic microcirculation, the development of ischemia and the onset and course of ANP. In our study TXB2 levels increased threefold, while 6-keto-PGF1alpha increased by a maximum of only 68% (at t=6 hours). Thus, it is possible that in ANP the balance between TXA2 and PGI2 is disturbed in favor of TXA2. Furthermore, some authors state that TXA2 exerts its adverse action indirectly by acting as a pharmacological inhibitor of PGI2 (7). Survival studies are in progress in our laboratory to measure the effect of changes in the TXA2/PGI2 ratio.

We also found slightly elevated 6-keto-PGF1alpha levels in rats pretreated with chloroquine (R) (group C). In addition, the PGE2 levels were significantly elevated in comparison to those found for group B (ANP) at three and six hours. The expected increase in TXB2 values was inhibited with significantly lower levels at three hours for group C compared to group B. Chloroquine (R) is considered to be a phospholipase A2 inhibitor (12). PLA2 blockers reportedly prolong survival in both experimental and clinical AP (15,16,31). It is possible that the beneficial action of PLA2 blockers can be attributed to inhibition of TXA2 formation; surprisingly it may also be associated with an elevation of the PGI2 and PGE2 levels. No dramatic effect on survival was observed for chloroquine (R) treatment in our study; only 30% of the animals survived. One explanation for this lack of a significant effect may be the fact that the TXA2/PGI2 ratio was changed only slightly in favor of PGI2.

The development of ANP also has an effect on PGE2 production. After induction of ANP a decrease in PGE2 levels was observed, a decrease that was progressive with time. At twelve hours the concentration was significantly lower compared to that found for the control group (A). At that time untreated animals started to die. Since the PGE series exerts important biological effects on several organs (1,8-10,33-35), the lower plasma levels may be important. The PGE series increases splanchnic blood flow and, according to some authors, also local blood flow (34). The PGE series is a group of vasodilators that act directly on vascular smooth muscle (1,34). These vasodilatory properties protect visceral organs from ischemic damage. PGE2 exhibits a cytoprotective action against ischemic splanchnic injury in the rat (36).

An interesting finding was a statistically significant increase in PGE2 levels compared to the control animals after treatment with FPL 55712 (group D). FPL 55712 is a leukotriene synthesis blocker (17). The significant increase in PGE2 levels suggests, indirectly, that leukotrienes are formed in ANP and that treatment with FPL 55712 shifts arachidonic acid from the lipoxygenase pathway toward the cyclooxygenase pathway. This so-called "redirection phenomenon" is not an uncommon event. For example, in vitro TXA2 inhibition is associated with a redirection of the metabolism of endoperoxides towards PGD2, PGE2 and PGI2 (32). The lipoxygenase pathway does not seem to play an essential role in ANP since only two out of nine animals survived

after pretreatment with FPL 55712.

The high levels of PGE2 in FPL 55712-pretreated animals did not improve survival time significantly. Possibly the relationship with other compounds of the arachidonic acid system is more important. In FPL 55712-pretreated animals the levels of TXB2 were high, while the 6-keto-PGF1alpha levels were low. Survival studies to clarify the role of PGE2 are in progress in our laboratory.

In summary, the results of the present studies indicate that arachidonate end-products play, at least in part, a role in acute necrotizing pancreatitis in rats. The significance of high TXB2 levels, low PGE2 levels and only slightly elevated 6-keto-PGF1alpha levels needs further investigation. The TXA2/PGI2 ratio may be of clinical importance.

7.6. REFERENCES

1. Robert A. Prostaglandins and digestive diseases. Adv Prostaglandin Thromboxane Res 1980;8:1533-1541.

2. Hyman AL, Mathe AA, Lippton HL, Kadowitz PJ. Prostaglandins and the lung. Med Clin North Am 1981;65:789-808.

3. Van Ooijen B, Ouwendijk RJT, Kort WJ, et al. Increased thromboxane B2 levels in acute necrotizing pancreatitis in rats. The effects of flunarizine, dazoxiben and indomethacin. Scand J Gastroenterol, accepted.

4. Bunting S, Moncada S, Vane JR. The prostacylin-thromboxane A2 balance: pathophysiological and therapeutic implications. Br Med Bull 1983;39:271-276.

5. Moncada S, Vane JR. Prostacyclin and its clinical applications. Ann Clin Res 1984;16:241-252.

6. Moncada S. Biological importance of prostacyclin. Br J Pharmacol 1982;76:3-31.

7. Lelcuk S, Alexander FA, Kobzik L, et al. Prostacyclin and thromboxane A2 moderate postischemic renal failure. Surgery 1985;98:207-212.

8. Tweedie JH, Mosley JG, Austin JL, Reber HA. Effect of 16,16-dimethyl prostaglandin E2 on aspirin-induced permeability changes in the pancreatic duct. Am J Surg 1981;141:227.

9. Wedgwood KR, Farmer RC, Reber HA. A model of hemorrhagic pancreatitis in cats - role of 16,16-dimethyl prostaglandin E2. Gastroenterology 1986;90:32-39.

10. Standfield NJ, Kakkar VV. Prostaglandins and acute pancreatitis - experimental and clinical studies. Br J Surg 1983;70:573-576.

11. Lankisch PG, Goke B, Kunze H, Otto J, Winckler K. Does PGE2 have a beneficial effect on acute experimental pancreatitis in the rat? Hepatogastroenterology 1983;36:148-150.

12. Nevalainen TJ. The role of phospholipase A in acute pancreatitis. Scand J Gastroent 1980;15:641-50.

13. Lands WEM. The biosynthesis and metabolism of prostaglandins. Ann Rev Physiol 1979;41:633-652.

14. Borgeat P, Samuelsson B. Metabolism of arachidonic acid in polymorphonuclear leukocytes. Structural analysis of novel hydroxylated compounds. J Biol Chem 1979;254:7865-7869.

15. Tykka H, Mahlberg K, Pantzar P, Tallberg T. Phospholipase A2 inhibitors and their possible clinical use in the treatment of acute pancreatitis. Scand J Gastroent 1980;15:519-528.

16. Schroder T, Lempinen M, Nordling S, et al. Chlorpromazine treatment of experimental acute fulminant pancreatitis in pigs. Eur Surg Res 1981;13:143-151.

17. Augstein J, Farmer JB, Lee TB, Sheard P, Tattersall ML. Selective inhibitor of slow reacting substance of anaphylaxis. Nature New Biol 1973;245:215-217.

18. Feuerstein G. Leukotrienes and the cardiovascular system. Prostaglandins 1984;27:781-802.

19. Hagmann W, Denzlinger C, Keppler D. Production of peptide leukotrienes in endotoxin shock. FEBS letters 2204 1985;180:309-313.

20. Ouwendijk RJT, Zijlstra FJ, Wilson JHP, et al. Raised plasma thromboxane B2 levels in alcoholic liver disease. Prostaglandins Leukotrienes Med 1983;10:115-122.

21. Warshaw AL, O'Hara PJ. Susceptibility of the pancreas to ischemic injury in shock. Ann Surg 1978;188:197-201.

22. Popper HC, Neckeles H, Russell KC. Transition of pancreatic edema into pancreatic necrosis. Surg Gyn Obstet 1948;87:79-82.

23. Moncada S, Vane JR. The role of prostacyclin in vascular tissue. Fed

Proc 1979;38:66-71.
24. Lelcuk S, Alexander F, Valeri CR, Shepro D, Hechtmann HB. Ischemia stimulate tissue thromboxane synthesis. Surg Forum 1984;35:76-78.
25. Webb PJ, Westwick J, Scully MF, et al. Do prostacyclin and thromboxane play a role in endotoxic shock? Br J Surg 1981;68:720-724.
26. Cook JA, Wise WC, Halushka PV. Elevated thromboxane levels in the rat during endotoxic shock. J Clin Invest 1980;65:227-230.
27. Carmona RH, Tanny TC, Trunkey DD. The role of prostacyclin and thromboxane in sepsis and septic shock. Arch Surg 1984;119:189-192.
28. Fink MP, MacVittie TJ, Casey LC. Inhibition of prostaglandin synthesis restores normal hemodynamics in canine hyperdynamic sepsis. Ann Surg 1984;200:619-626.
29. Harris RH, Zmudka MI, Maddox Y, et al. Relationship of TXB2 and 6-keto-PGF1alpha to the hemodynamic changes during baboon endotoxic shock. Adv Prostaglandin Thromboxane Res 1980;7:843-849.
30. Casey LC, Fletcher JR, Zmudka MI, Ramwell PW. Prevention of endotoxin-induced pulmonary hypertension in primates by the use of a selective thromboxane synthetase inhibitor, OXY 1581. J Pharm Exp Ther 1982;222:441-446.
31. Tykka H, Vaittinen EJ, Mahlberg KL, et al. A randomized double-blind study using CaNa2EDTA, a phospholipase A2 inhibitor, in the management of human acute pancreatitis. Scand J Gastroenterol 1985;20:5-12.
32. O'Keefe EH, Liu ECK, Greenberg R, Ogletree ML. Effects of a thromboxane synthetase inhibitor and a thromboxane antagonist on release and activity of TXA2 and prostacyclin in vitro. Prostaglandins 1985;29:785-797.
33. Homma T, Malik KU. Effect of prostaglandins on pancreatic circulation in anesthetized dogs. J Pharmacol Exp Ther 1981;222:623-628.
34. Tobimatsu M, Konomi K, Saito S, Tsumagari T. Protective effect of prostaglandin E1 on ischemia-induced acute renal failure in dogs. Surgery 1985;98:45-52.
35. Arendshorst WJ, Johnston PA, Selkurt EE. Effect of prostaglandin E on renal hemodynamics in nondiuretic and volume-expanded dogs. Am J Physiol 1974;226:218-225.
36. Dawiskiba J, Isaksson B, Jeppson B, Hagerstrand I, Bengmark S. Cytoprotective effect of PGE2 on ischemic splanchnic injuries in the rat. Eur Surg Res 1984;16:77-83.

CHAPTER 8

THE SIGNIFICANCE OF PROSTAGLANDIN E2 IN ACUTE NECROTIZING PANCREATITIS IN RATS.

Bart van Ooijen (1), Wil J Kort (1), Cor J Tinga (2),
JH Paul Wilson (3), Dick L Westbroek (1).

Departments of Experimental Surgery (1) and Internal Medicine (3), Faculty of Medicine, Erasmus University, Rotterdam; Department of Pathology (2), Bronovo Hospital, The Hague.

8.1. ABSTRACT

Administration of PGE2 to rats with acute necrotizing pancreatitis (ANP) decreased the mortality rate from 100% at 36 hours to 60%. ANP was induced by injecting 5% sodium taurocholate into the pancreatic duct. PGE2 was administered twice in a dose of 100 mu-gram/kg subcutaneously. A further increase in survival was achieved by pretreatment with either dazmegrel (R), a thromboxane A2 synthesis inhibitor, or flunarizine (R), a calcium entry blocker that is known to inhibit TXA2 formation. After flunarizine (R) and PGE2 pretreatment 60% of the animals survived for 72 hours ($p < 0.05$). Since plasma TXB2 (the stable metabolite of TXA2) levels increase and the plasma PGE2 levels decrease in ANP, the results of the present study indicate that these prostaglandins play a role in the pathophysiology of this disease. It is suggested that restoration of the balance between prostanoid levels will have a beneficial effect on the course of ANP.

8.2. INTRODUCTION

Several reports have demonstrated that prostaglandins protect visceral organs against a variety of noxious agents (1-3). However, the effect of prostaglandins on the pancreas in acute necrotizing pancreatitis (ANP) remains controversial (4-9). Ischemia and impairment of the pancreatic microcirculation may play an important role in the onset and course of ANP

(10,11). We have shown that thromboxane B2 (TXB2) levels are elevated in ANP (12,13). Thromboxane A2 (TXA2) is a strong vasoconstrictor and as such may be a mediator of ischemia. However, inhibition of TXA2 alone does not dramatically alter survival in a rat model (12). We have also demonstrated that plasma levels of prostaglandin E2 decrease in ANP (13). Prostaglandin E2 (PGE2) has vasodilatory properties that may protect visceral organs against ischemic damage (1); it is also reported to have cytoprotective properties that guard against ischemic splanchnic injury in the rat (14). As such PGE2 may play a protective role in ANP. Acute necrotizing pancreatitis with its low PGE2 and high TXA2 levels has a 100% mortality rate (12,13). Administration of FPL 55712 (a 5-lipoxygenase inhibitor) to rats with ANP was associated with high PGE2 levels which, even in the presence of high TXA2 levels, reduced mortality by 22% (13). However the administration of exogenous PGE2 alone did not affect survival significantly (7,9). Our previous experiments led us to test the effects of PGE2 administration on survival when TXA2 synthesis was inhibited simultaneously.

We tested the effects of PGE2 in animals with ANP and in animals with ANP that were pretreated with either dazmegrel, a TXA2 synthetase blocker (15), or flunarizine (R), a calcium entry blocker. Flunarizine decreases TXA2 formation (12) and is also known to inhibit the effects of elevated TXA2 levels (16).

8.3. MATERIALS AND METHODS

Male Wag/Rij rats, weighing 200-250 grams, were used in the studies. Acute necrotizing pancreatitis was induced by retrograde injection of 5% sodium taurocholate (STC) (0.1 ml/100 g body weight) into the biliopancreatic duct, as previously described (12).

Prostaglandin E2 (PGE2) (lot 54F - 0110; Sigma) was stored at -30 degrees C in absolute ethanol; final dilutions in physiological saline (50 mu-gram/ml) were prepared on the day of injection. The PGE2 injections (100 mu-gram/kg body weight) were given subcutaneously at the time of the induction of ANP and six hours later.

Dilutions of dazmegrel (UK no. 38485 lot no. R22; Pfizer Central Research Laboratories, Sandwich, England) in 0.1 n NaOH (12.5 mg/ml) were prepared on the day of administration. Test animals received a dose of 50 mg/ml dazmegrel via an intragastric tube one hour prior to the induction of ANP and 12 hours

later.

Flunarizine (R) was obtained as a gift from Janssens Pharmaceuticals, Goirle, the Netherlands. Dilutions in physiological saline (0.1 mg/ml) were prepared on the day of injection. Test animals received one single intravenous injection (0.2 mg/kg body weight) at the time of the induction of ANP. The rats were randomly assigned to one of four groups.

group 1 (8 rats): Control group. ANP was induced and saline (0.5 ml) administered subcutaneously at zero time and six hours later.

group 2 (10 rats): Test group. ANP was induced and PGE2 was administered twice.

group 3 (10 rats): Test group. ANP was induced and both PGE2 and dazmegrel were administered twice.

group 4 (10 rats): Test group. ANP was induced; PGE2 was administered twice and flunarizine once.

Survival time was recorded and survivors were sacrificed after 72 hours. The amount of ascitic fluid was measured at the time of autopsy by weighing cotton rolls saturated with the fluid. The pancreas was removed, fixed in 4% formalin and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin. The preparations were assessed by light microscopy.

The amounts of ascitic fluid for the different groups were compared using the Mann Whitney U test, the survival data using Fischer's test for 2x2 tables.

8.4. RESULTS

The effect of treatment with PGE2 on mortality for rats with acute necrotizing pancreatitis is shown in table 1.

Control animals (group 1) exhibited a 100% mortality. Treatment with two doses of 100 mu-gram/kg PGE2 (group 2) reduced mortality to 60%, an effect that was enhanced by adjuvant treatment with either flunarizine (group 3) or dazmegrel (group 4). According to Fischer's test, a significant difference for groups 3 and 4 with the control group was obtained in the first 36 hours, when

the mortality rate was 40% ($p < 0.05$). One animal of the dazmegrel/PGE2 group died after 36 hours.

Table 1. Mortality rate for acute necrotizing pancreatitis in rats.

	time period		
group	0-36	36-72	0-72
AP	100	0	100
AP+PGE2	60	0	60
AP+PGE2+D	40*	10	50
AP+PGE2+F	40*	0	40*

The mortality rate is expressed as the percentage animals that died in each time period. AP=acute pancreatitis; D=dazmegrel; F=flunarizine.
* $p < 0.05$ statistically significant with respect to the AP group (Fischer's test for 2 x 2 tables).

Ascites developed in all animals that died within 72 hours. The largest amounts of ascitic fluid were found in rats that died in the first 36 hours. The volume of ascitic fluid was not decreased by treatment with PGE2, when the rats were grouped according to survival time. However, after additional pretreatment with dazmegrel the decrease in fluid was statistically significant ($p < 0.05$) (table 2).

Light microscopy studies of the pancreas showed inflammatory infiltration with large areas of acinar necrosis. The inflammatory infiltrate was not pronounced in the first 24 hours, but became severe at 72 hours. Apparently, necrosis continued since at 72 hours sometimes more than 80% of the pancreas was necrotic. Acinar necrosis was less pronounced in the dazmegrel/PGE2 group, because usually less than 50% necrosis was observed (table 3).

In the animals that survived for 72 hours bowel and liver were attached to the pancreas. Hydrothorax developed in some of the animals that died in the first 24 hours. In some instances a distended stomach with signs of a paralytic ileus of the proximal bowel was found. Fat necrosis was pronounced 24 hours after induction and tended to increase with time. At 72 hours severe

fat necrosis throughout the abdominal cavity was observed in all animals.

Table 2. Amount of ascitic fluid produced in acute necrotizing pancreatitis.

	time period	
group	0-36 hours	36-72 hours
AP	7.6±1.4 (8)	-
AP+PGE2	8.9±2.2 (4)	0.6±0.4 (4)
AP+PGE2+F	5.8±2.9 (3)	1.3±0.6 (6)
AP+PGE2+D	3.3±1.0 (4) *	0.8±0.9 (6)

The values, given in grams of ascitic fluid, are expressed as means ± SD. The number of animals is indicated in parentheses. AP=acute pancreatitis; D=dazmegrel; F=flunarizine. * $p < 0.05$ significant with respect to control values (Mann Whitney U test).

Table 3. Amount of necrosis in different groups with acute necrotizing pancreatitis.

group	amount of necrosis in % *							
	0-36 hours				36-72 hours			
	% <10	10-25	25-50	>50	<10	10-25	25-50	>50
AP			4	4				
AP+PGE2	3		1	1				3
AP+PGE2+F	2	1						5
AP+PGE2+D		3		1	1	1	3	1

*The amount of necrosis was expressed as a percentage of the whole gland on the basis of a double blind microscopic evaluation of 34 slides. The number of slides is indicated. AP=acute pancreatitis; D=dazmegrel; F=flunarizine.

8.5. DISCUSSION

The present study demonstrates the effects of the administration of exogenous prostaglandin E2 to rats with acute necrotizing pancreatitis. Previously, we reported decreased plasma levels of PGE2 in ANP (13). In this study we show that supplementation of PGE2 with exogenous PGE2 led to a decrease in the mortality rate from 100% to 60%.

The role of the PGE series in pancreatic physiology and disease has so far been controversial (1,17). A protective effect of PGE1 or PGE2 in AP has been reported by Manabe (4), Standfield (5), Coelle (6) and Reber (8), but a lack of effect was observed by Martin (9), Lankisch (7), Crocket (18) and Olazabal (19), while a deleterious effect was demonstrated in a study by Wedgwood et al. (20). However, these studies were not comparable as far as the (animal) model, the PGE analogue and the dosage of PGE are concerned. For example Manabe (4), Standfield (5) and Martin (9) chose a diet model in mice but administered different dosages of PGE2. Crocket (18) used the closed duodenal loop model to induce AP in rats and administered PGE1. Using the same model of ANP as described in the present study, Lankisch (7) could not show any beneficial effect, while Reber (8) initially found a protective effect which he could not confirm in later studies with cats. Differences in experimental design might, at least partially, explain the discrepancies between results. It is still not known which dose of PGE2 will reach the inflamed pancreas in a sufficiently high concentration when given subcutaneously.

The mode of action of PGE in ANP is not clear. PGE's are vasodilators which act directly on vascular smooth muscle (1). These vasodilatory properties may protect visceral organs from ischemic damage. PGE2 has a protective effect against ischemic splanchnic injury in the rat model (14). PGE2 may increase local blood flow by reducing vascular resistance (21,22), and may also increase vascular permeability (20). PGE2 may function as a local counter-regulatory hormone modulating sympathetic tone (23). It is possible that PGE2 acts at sites other than the pancreas. PGE2 may, by cytoprotection, prevent both local and systemic release of activated pancreatic digestive enzymes (5).

Among the factors associated with vasoconstriction are TXA2 and other vasoactive mediators (24). TXA2 stimulates platelet aggregation leading to the formation of microthrombi, which are detrimental to the microcirculation (25).

In contrast PGE2 inhibits platelet aggregation (24). Thus, the effects of PGE2 may be antagonized by elevated TXA2 levels.

We reported in a previous study that the use of a TXA2 synthetase blocker alone does not alter the survival rate for ANP significantly (12). In the present study treatment with a thromboxane A2 synthetase blocker (dazmegrel) together with the administration of exogenous PGE2 led to a significant improvement in survival rate in the first 36 hours ($p < 0.05$). Pretreatment of test animals with flunarizine and PGE2 caused a significant improvement in the survival rate at 72 hours ($p < 0.05$). Flunarizine is a calcium entry blocker and probably acts as a phospholipase A2 inhibitor (12,26). TXA2 levels are decreased by flunarizine, as previously reported (12); however treatment with flunarizine alone does not alter survival time (12).

Calcium entry blockers may influence the formation of prostacyclin as well (27). An increase in PGI2 synthesis in addition to inhibition of platelet aggregation was seen in experiments with the calcium blocker diltiazem (R) (27). PGE2, on the one hand, and inhibition of TXA2 and stimulation of PGI2 by flunarizine, on the other, are possibly responsible for the observed beneficial effect of this combined treatment on survival in ANP. The present results suggest that among other things both inhibition of vasoconstriction and stimulation of vasodilation are important for survival in ANP.

Autopsy results seem to indicate that the condition of the pancreas itself may not be of exclusive importance for rat survival. Most animals died within 24 hours at which time both the inflammatory reaction in the pancreas and necrosis were not pronounced. However, at 72 hours necrosis and the inflammatory reaction were severe in animals that survived. Shock and fluid loss probably played a role and the accumulation of ascitic fluid was severe in the first 36 hours. Therapy with PGE2 did not influence the amount of ascitic fluid formed. However, simultaneous administration of a TXA2 blocker significantly lowered the amount of ascitic fluid.

In summary, the results of this study indicate that PGE2 plays a role in ANP. Administration of PGE2 increases the survival rate, especially when TXA2 synthesis is inhibited.

8.6. REFERENCES

1. Robert A. Prostaglandins and digestive diseases. Adv Prostaglandin Thromboxane Res 1980;8:1533-1541.

2. Hyman AL, Mathe AA, Lippton HL, Kadowitz PJ. Prostaglandins and the lung. Med Clin North Am 1981;65:789-808.

3. Miller P, Fischer N, Damann HG, Kather H, Simon B. The cytoprotective effect of 16,16-dimethyl prostaglandin E2 on bile salt induced damage to the human stomach. Br J Clin Pharmacol 1981;12:253-254.

4. Manabe T, Steer ML. Protective effects of PGE2 on diet induced acute pancreatitis in mice. Gastroenterology 1980;78:777-781.

5. Standfield NJ, Kakkar VV. Prostaglandins and acute pancreatitis - experimental and clinical studies. Br J Surg 1983;70:573-576.

6. Coelle EF, Adham N, Elashoff J, Lewin K, Taylor IL. Effects of prostaglandin and indomethacin on diet-induced acute pancreatitis in mice. Gastroenterology 1983;85:1307-1312.

7. Lankisch PG, Goke B, Kunze H, Otto J, Winckler K. Does PGE2 have a beneficial effect on acute experimental pancreatitis in the rat? Hepatogastroenterology 1983;36:148-150.

8. Reber HA, Tweedie JH, Mosely JG. The cytoprotective effect of 16,16-dimethyl prostaglandin E2 on bile salt induced damage to the pancreas. Gastroenterology 1980;78:1241.

9. Martin DM, Someren AO, Nasrallah SM. The value of prostaglandin E2 in ethionine induced pancreatitis. Gastroenterology 1981;80:1224.

10. Warshaw AL, O'Hara PJ. Susceptibility of the pancreas to ischemic injury in shock. Ann Surg 1978;188:197-201.

11. Popper HC, Neckeles H, Russell KC. Transition of pancreatic edema into pancreatic necrosis. Surg Gyn Obstet 1948;87:79-82.

12. Van Ooijen B, Ouwendijk RJT, Kort WJ, et al. Increased thromboxane B2 levels in acute necrotizing pancreatitis in rats. The effects of flunarizine, dazoxiben and indomethacin. Scand J Gastroenterol, accepted.

13. Van Ooijen B, Kort WJ, Zijlstra FJ, Vincent JE, Wilson JHP, Westbroek DL. Prostanoid imbalance in experimental acute necrotizing pancreatitis in rats. Scand J Gastroenterol, accepted.

14. Dawiskiba J, Isaksson B, Jeppson B, Hagerstrand I, Bengmark S. Cytoprotective effect of PGE2 on ischemic splanchnic injuries in the rat. Eur Surg Res 1984;16:77-83.

15. Fischer S, Struppler M, Bohlig B, Bernutz C, Wober W, Weber PC. The influence of selective thromboxane synthetase inhibition with a novel imidazole derivative, UK-38485, on prostanoid formation in man. Circulation 1983;68:821-826.

16. De Clerck F, Van Nueten JM. Platelet-mediated vascular contraction. Inhibition by flunarizine, a calcium entry blocker. Bioch Pharmacol 1983;32:765-771.

17. Wilson DE, Kaymakcalan H. Prostaglandins: Gastrointestinal effects and peptic ulcer disease. Med Clin North Am 1981;65:773-787.

18. Crocket KV, Brackett K, Crocket A, Jacobson ED, Rao SS, Joffe SN. Prostaglandin E1 treatment in experimental acute pancreatitis in the rat. Eur Surg Res 1984;16:265-273.

19. Olazabal A. Effect of prostaglandin E2 and I2 and of indomethacin on deoxycholic acid-induced damage to the rat bile-pancreatic duct. Gastroenterology 1983;84:928-934.

20. Wedgwood KR, Farmer RC, Reber HA. A model of hemorrhagic pancreatitis in cats - role of 16,16-dimethyl prostaglandin E2. Gastroenterology 1986;90:32-39.

21. Homma T, Malik KU. Effect of prostaglandins on pancreatic circulation in anesthetized dogs. J Pharmacol Exp Ther 1981;222:623-628.
22. Saunders RN, Moser CA. Changes in vascular resistance induced by prostaglandins E2 and F2 in the isolated rat pancreas. Arch Int Pharmacodyn 1972;197:86-92.
23. Fink MP, MacVittie TJ, Casey LC. Inhibition of prostaglandin synthesis restores normal hemodynamics in canine hyperdynamic sepsis. Ann Surg 1984;200:619-626.
24. Ouwendijk RJT. Eicosanoids, endotoxins and liver disease. Thesis. Erasmus University. Rotterdam, 1985.
25. Webb PJ, Westwick J, Scully MF, et al. Do prostacyclin and thromboxane play a role in endotoxic shock? Br J Surg 1981;68:720-724.
26. Nevalainen TJ. The role of phospholipase A in acute pancreatitis. Scand J Gastroent 1980;15:641-650.
27. Mehta J, Mehta P, Ostrowski N. Calcium blocker diltiazem inhibits platelet activation and stimulates vascular prostacyclin synthesis. Am J Med Sci 1986;291:20-24.

CHAPTER 9

THE SIGNIFICANCE OF THE THROMBOXANE A2 - PROSTAGLANDIN I2 RATIO IN ACUTE NECROTIZING PANCREATITIS IN RATS.

Bart van Ooijen (1), Wil J Kort (1), Cor J Tinga (2), JH Paul Wilson (3), Dick L Westbroek (1).

Departments of Experimental Surgery (1) and Internal Medicine (3), Faculty of Medicine, Erasmus University, Rotterdam; Department of Pathology (2), Bronovo Hospital, The Hague.

9.1. ABSTRACT

Administration of iloprost (ZK 36374, a stable analogue of PGI2) to rats with acute necrotizing pancreatitis (ANP) decreased the mortality rate from 100% at 36 hours to 50%. ANP was induced by injecting 5% sodium taurocholate into the pancreatic duct. Iloprost was administered subcutaneously in a dose of 25 ng/kg body weight at zero time and 1,2,3,6 and 9 hours after induction of ANP. An additional increase in survival was achieved by pretreatment with either flunarizine (R), a calcium entry blocker that is known to inhibit TXA2 formation and effects, or dazmegrel, a thromboxane A2 synthesis blocker. Sixty per cent survived for more than 36 hours ($p < 0.05$) after treatment with flunarizine and iloprost and 90% when dazmegrel and iloprost were given ($p < 0.01$). The results of the present study indicate that the ratio between TXA2 and PGI2 is important in ANP. Administration of iloprost has a beneficial effect on the course of ANP, which can be further improved by simultaneous inhibition of TXA2 synthesis.

9.2. INTRODUCTION

Prostaglandins play an important role in the pathophysiology of several diseases (1-3). They possess potent and diverse biological activities. Recently we demonstrated the presence of a prostanoid imbalance in acute

necrotizing pancreatitis (ANP) (4). Thromboxane A2 (TXA2) is a potent vasoconstrictor and a stimulus of platelet aggregation (1-3). Plasma levels of thromboxane B2 (TXB2), the stable end-product of TXA2, were elevated in ANP (4,5).

Prostacyclin (PGI2), on the other hand, is a vasodilator and the most potent naturally occurring inhibitor of platelet aggregation yet discovered (6,7). The levels of 6-keto PGF1alpha, the stable product of PGI2, are also elevated in ANP, although the TXB2 levels tend to rise more dramatically than those of 6-keto-PGF1alpha (4).

These differences in -sometimes opposite- biological activities have led to the development of new concepts in vascular homeostasis (8). In this respect, several reports stress the importance of the thromboxane A2/prostaglandin I2 ratio (6,8). In the present study we investigated the effect of altering the TXA2/PGI2 ratio on survival and histology in ANP. For this purpose iloprost (ZK 36 374, a stable PGI2 derivate) (9), was administered to animals with ANP and animals with ANP in which TXA2 synthesis was inhibited. The synthesis of TXA2 was inhibited by dazmegrel (R), a thromboxane A2 synthesis inhibitor (10), and flunarizine (R), a calcium entry blocker (11). Flunarizine decreases TXA2 formation, as previously reported (5), and also inhibits the effects of elevated TXA2 levels (11).

9.3. MATERIALS AND METHODS

Inbred male Wag/Rij rats, weighing 200-250 grams, were used. Acute necrotizing pancreatitis was induced by the retrograde injection of 5% sodium taurocholate (STC) (0.1 ml/100 g body weight) into the biliopancreatic duct, as previously described (5).

Iloprost (ZK 36374) vials containing 1 ml of injection fluid no. SH L 401 A, were a gift from Schering AG, Berlin, West Germany. Final dilutions in physiological saline (10 ng/ml) were prepared on the day of injection. Test animals were injected subcutaneously with iloprost at the time of induction of ANP (zero time) and 1,2,3,6 and 9 hours after induction in a dose of 25 ng/kg body weight. Control animals received 0.9% NaCl instead of iloprost.

Dilutions of dazmegrel (UK no. 38485 lot no. R22; Pfizer Central Research Laboratories, Sandwich, England) in 0.1 n NaOH (12.5 mg/ml) were prepared on the day of administration. Experimental animals received a dose of 50 mg/kg

body weight dazmegrel via a gastric tube one hour prior to the induction of ANP and 12 hours later.

Flunarizine (R) was a gift of Janssens Pharmaceuticals, Goirle, the Netherlands. Dilutions in 0.9 % NaCl (0.1 mg/ml) were prepared on the day of injection. Experimental animals were given flunarizine intravenously at the time of the induction of ANP in a dose of 0.2 mg/kg body weight.

The rats were randomly assigned to one of four groups.

group 1 (8 rats) : Control group. ANP was induced and saline (0.5 ml) administered subcutaneously at zero time and 1,2,3,6 and 9 hours after induction of ANP.

group 2 (8 rats) : Test group. ANP was induced and 6 dosages of iloprost were administered.

group 3 (10 rats): Test group. ANP was induced and 6 dosages of iloprost and 2 dosages of dazmegrel were administered.

group 4 (10 rats): Test group. ANP was induced and 6 dosages of iloprost and one single dose of flunarizine were administered.

Survival time was recorded and survivors were sacrificed after a 72-hour observation period. Ascites was measured at autopsy by weighing cotton rolls saturated with the ascitic fluid. The pancreas was removed, fixed in 4% buffered formalin and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin. The preparations were assessed by light microscopy.

The amounts of ascitic fluid for the different groups were compared using the Mann Whitney U test, the survival data using Fischer's test for 2x2 tables.

9.4. RESULTS

The effect of treatment with iloprost on the mortality rate for rats with acute necrotizing pancreatitis is shown in table 1. Control animals exhibited a 100% mortality at 36 hours. Treatment with iloprost (25 ng/kg body weight)

reduced mortality to 50%. An even better result was obtained after pretreatment of the animals with flunarizine (R) (40% mortality) ($p < 0.05$) and when dazmegrel was given in addition to iloprost 90% of the animals survived for 36 hours ($p < 0.01$).

Table 1. Mortality rate for rats with acute necrotizing pancreatitis.

	time period		
group	0-36	36-72	0-72
AP	100	0	100
AP+iloprost	50	0	50
AP+iloprost+F	40*	0	40*
AP+iloprost+D	10**	10	20**

The mortality rate is expressed as the percentage animals that died in the given time period. AP=acute pancreatitis; D=dazmegrel; F=flunarizine. * $p < 0.05$ statistically significant with respect to the AP group ** $p < 0.01$ statistically significant with respect to the AP group (Fischer's test for 2 x 2 tables).

Ascites developed in all animals that died within 72 hours. The largest amounts of ascitic fluid were found in the first 36 hours. The amount of ascitic fluid decreeased significantly after treatment with iloprost even when the rats were grouped according to survival time. Moreover, pretreatment with dazmegrel reduced the ascites even more ($p < 0.05$). Results are summarized in table 2.

Light microscopy studies of the pancreas showed an inflammatory infiltrate with large areas of acinar necrosis. Inflammatory infiltration was not pronounced in the first 24 hours, but became severe at 72 hours. Moreover, necrosis apparently continued since at 72 hours more than 80% of the pancreas was necrotic in some cases. Acinar necrosis was less pronounced in the dazmegrel/PGI2 group, because usually less than 50% necrosis was observed (table 3).

Table 2. Amount of ascitic fluid produced in acute necrotizing pancreatitis.

time period / group	0-36 hours	36-72 hours
AP	6.4±1.5 (8)	--
AP+iloprost	3.3±1.0 (4) *	1.5±1.0 (4)
AP+iloprost+F	4.5±1.2 (4)	0.3±0.3 (6)
AP+iloprost+D	5.0 (1)	0.9±0.8 (9)

The values, given in grams of ascitic fluid, are expressed as means ± SD. The number of animals is indicated in parentheses. AP=acute pancreatitis; D=dazmegrel; F=flunarizine. * $p < 0.05$ significant with respect to the control values (Mann Whitney U test).

Table 3. Amount of necrosis in different groups of rats with acute necrotizing pancreatitis.

group	amount of necrosis in % *							
	0-36 hours				36-72 hours			
	% <10	10-25	25-50	>50	<10	10-25	25-50	>50
AP			4	4				
AP+ilo	1		1	1			1	3
AP+ilo+F			3	1			1	4
AP+ilo+D				1			5	3

*The amount of necrosis, expressed as a percentage of the whole gland, is based on a double blind microscopic evaluation of 33 slides. The number of slides is indicated. AP=acute pancreatitis; ilo = iloprost; D = dazmegrel; F = flunarizine.

In the animals that survived for 72 hours the pancreas was attached to bowel and liver. Hydrothorax developed in some of the animals that died in the first 24 hours. In several cases a distended stomach with signs of a paralytic ileus of the proximal bowel was found. Fat necrosis was seen 24 hours after induction and tended to increase with time. At 72 hours severe fat necrosis throughout the abdominal cavity was observed in all animals except those pretreated with flunarizine; in the latter the fat necrosis was very mild throughout the abdominal cavity.

9.5. DISCUSSION

Thromboxane A2 and prostacyclin are products of arachidonic acid with different and in many respects opposite biological activities. They play an important role in vascular homeostasis (8). The interaction between platelet and vessel wall is responsible for eicosanoid production (3,8). TXA2 is produced mainly by platelets, but also by leukocytes, macrophages, mast cells and human fibroblasts (3), in response to ischemia in particular (12). The main effect of TXA2 on the circulation is constriction of blood vessels and stimulation of platelet aggregation. As such it plays an important role in the macro and microcirculations of various organs. PGI2 is a strong vasodilatory agent that dilates all vascular beds (13), and is the most powerful naturally occurring inhibitor of platelet aggregation known (6-8). It is produced in the walls of veins and arteries. PGI2 inhibits TXA2 production by elevating the cAMP concentration in platelets (6). The increase in cAMP causes inhibition of platelet phospholipase (14,15) as well as platelet cyclooxygenase (16). A precise balance between TXA2 and PGI2 seems to be important for vascular homeostasis (8,17,18).

TXA2 is generated in ANP, as reported previously (4,5). Plasma 6-keto-PGF1alpha levels are increased as well, although to a lesser extent than the TXA2 levels (4). In the present series of experiments we tried to elucidate the importance of changes in the ratio between TXA2 and PGI2 in ANP to survival.

Administration of iloprost, a stable analogue of PGI2, to rats with ANP lowered mortality from 100% to 50% at 36 hours. A dramatic improvement in survival was seen when a combination of a TXA2 synthesis blocker and exogenous iloprost was given. Inhibition of TXA2 synthesis with dazoxiben without

exogenous iloprost tends to improve survival from 0% to 40% at 36 hours (5). Dazoxiben is known to cause a slight redirection of TXA2 to PGI2 in vitro, although such an effect has as yet not been demonstrated in vivo (10,19).

The present results show that TXA2 and PGI2 play a role in ANP. Their balance could be important for local vascular homeostasis. Pancreatic blood flow decreases profoundly during the first hours of an episode of acute necrotizing pancreatitis (20-23). Edema, poor vascular filling and spastic changes in the lobular vessels have been observed (21). PGI2 dilates the splanchnic circulation, thereby improving the blood flow (13,24). In contrast, TXA2 activity leads to vasoconstriction and the formation of microthrombi due to platelet aggregation, both of which are detrimental to the microcirculation.

We also tested the effects of iloprost on flunarizine-pretreated animals. Flunarizine is a calcium entry blocker (11). Flunarizine lowers the levels of TXA2 in ANP (5), but does not in itself improve survival significantly . However, in combination with iloprost a significant improvement in survival was achieved. As reported previously, flunarizine pretreatment in combination with PGE2 therapy increases the survival rate for rats with ANP significantly from 0% to 60% at 36 hours (25). Since calcium entry blockers not only inhibit platelet activation but also stimulate vascular prostacyclin synthesis (26), the significant increase in survival rate obtained with flunarizine in combination with either PGE2 or iloprost may be due to this effect of the drug.

Histological examination of the pancreas showed that there was relatively less necrosis in pretreated animals. The smallest percentage necrosis was seen in dazmegrel/iloprost-pretreated animals (table 3), which might be attributable to a favorable effect on the pancreatic microcirculation whereby cell death is prevented or an effect on the pancreatic cell itself.

Animals that died within 24 hours did not exhibit as much necrosis as the animals sacrificed at 72 hours. At 72 hours necrosis and the inflammatory reaction were more severe, but these animals had survived. These findings suggest that local lesions of the pancreas are not exclusive factors of survival.

Ascites, pleural effusions and ileus were seen in the animals that died in the first 24 hours. Less ascitic fluid had accumulated in iloprost-treated animals. Blockage of eicosanoid synthesis (including PGI2) is reported to be associated with an increased volume of ascitic fluid in pigs with AP (27). Since PGI2 causes dilatation of the vascular bed and an increase in the

vascular permeability, the smaller amounts apparently cannot be explained by the vascular properties of PGI2.

No ascites was found after 72 hours and the pancreas was cut off from the peritoneal cavity by a large infiltrate. It is possible that the balance between TXA2 and PGI2 is important for maintenance of a sufficient blood flow to vital organs until the large fluid loss into the peritoneal cavity stops.

PGI2 may reduce the formation of activated pancreatic enzymes and their release into the systemic circulation, because PGI2 is known to decrease the formation and release of lysosomal hydrolases, also in infarcted areas (6,7). Lysosomal hydrolases are thought to play a role in the intracellular activation of proteolytic enzymes (28). PGI2 exhibits a cytoprotective effect on pancreatic lysosomes in less advanced acute pancreatitis in dogs (29). This cytoprotective effect may be a consequence of the local vascular effects of PGI2, because lysosomes are sensitive to stimuli such as ischemia, hypoxia and acidosis (30). A protective effect of PGI2 on hepatic, renal and pulmonary lysosomes is also reported to occur during an attack of AP in dogs (31-33). These organs are frequently damaged during the course of ANP.

In summary, the results of this study indicate that the TXA2/PGI2 ratio is important in ANP. Administration of iloprost has a beneficial effect on the course of ANP, especially when simultaneous inhibition of TXA2 synthesis is achieved.

9.6. REFERENCES

1. Robert A. Prostaglandins and digestive diseases. Adv Prostaglandin Thromboxane Res 1980;8:1533-1541.

2. Goerig M, Habenicht AJR, Schettler G. Eicosanoide und phospholipasen. Klin Wochenschr 1985;63:293-311.

3. Ouwendijk RJT. Eicosanoids, endotoxins and liver disease. Thesis. Erasmus University. Rotterdam, 1985.

4. Van Ooijen B, Kort WJ, Zijlstra FJ, Vincent JE, Wilson JHP, Westbroek DL. Prostanoid imbalance in experimental acute necrotizing pancreatitis in rats. Scand J Gastroenterol, accepted.

5. Van Ooijen B, Ouwendijk RJT, Kort WJ, et al. Increased thromboxane B2 levels in acute necrotizing pancreatitis in rats. The effects of flunarizine, dazoxiben and indomethacin. Scand J Gastroenterol, accepted.

6. Moncada S, Vane JR. Prostacyclin and its clinical applications. Ann Clin Res 1984;16:241-252.

7. Moncada S. Biological importance of prostacyclin. Br J Pharmacol 1982;76:3-31.

8. Bunting S, Moncada S, Vane JR. The prostacylin-thromboxane A2 balance: pathophysiological and therapeutic implications. Br Med Bull 1983;39:271-276.

9. Krause W, Schubert M. Pharmacokinetics and biotransformation of the stable prostacyclin analogue ZK 36 374. II. Blood and plasma levels and passage of the blood brain barrier in the rat. Eur J Drug Metab Pharmacokinet 1983;8:145-150.

10. Fischer S, Struppler M, Bohlig B, et al. The influence of selective thromboxane synthethase inhibition with a novel imidazol derivate, UK-38485, on prostanoid formation in man. Circulation 1983;68:821-826.

11. De Clerck F, Van Nueten JM. Platelet-mediated vascular contraction. Inhibition by flunarizine, a calcium entry blocker. Bioch Pharmacol 1983;32:765-771.

12. Lelcuk S, Alexander F, Valeri CR, Shepro D, Hechtmann HB. Ischemia stimulate tissue thromboxane synthesis. Surg Forum 1984;35:76-78.

13. Moncada S, Vane JR. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A2 and prostacyclin. Pharmacol Rev 1978;30:293-331.

14. Lapetina EG, Smitges CJ, Chandrabose K, Cuatrecasas P. Cyclic adenosine 3',5'-monophosphate and prostacyclin inhibit membrane phospholipase activity in platelets. Biochem Biophys Res Commun 1977;76:828-835.

15. Minkes M, Stanford M, Chi M, et al. Cyclic adenosine 3'5'-monophosphate inhibits the availability of arachidonate to prostaglandin synthethase in human platelet suspensions. J Clin Invest 1977;59:449-454.

16. Malmsten C, Granstom E, Samuelsson B. Cyclic AMP inhibits synthesis of prostaglandin peroxide (PGG2) in human platelets. Biochem Biophys Res Commun 1976;68:569-576.

17. Webb PJ, Westwick J, Scully MF, et al. Do prostacyclin and thromboxane play a role in endotoxic shock? Br J Surg 1981;68:720-724.

18. Carmona RH, Tanny TC, Trunkey DD. The role of prostacyclin and thromboxane in sepsis and septic shock. Arch Surg 1984;119:189-192.

19. O'Keefe EH, Liu ECK, Greenberg R, Ogletree ML. Effects of a thromboxane synthetase inhibitor and a thromboxane antagonist on release and activity of TXA2 and prostacyclin in vitro. Prostaglandins 1985;29:785-797.

20. Papp M, Makara GB, Hajtman B, et al. A quantitative study of pancreatic blood flow in experimental pancreatitis. Gastroenterology 1966;51:524-528.

21. Schiller WR, Anderson MC. Microcirculation of the normal and inflamed canine pancreas. Ann Surg 1975;181:466-470.

22. Goodhead B. Acute pancreatitis and pancreatic blood flow. Surg Gyn

Obstet 1969;69:331-340.
23. Donaldson LA. Pancreatic circulation reduces rapidly in acute pancreatitis. Surgery 1978;84:313-321.
24. Lefer AM, Tabas J, Smith EF. Salutary effects of prostacyclin in endotoxic shock. Pharmacology 1980;21:206-212.
25. Van Ooijen, Kort WJ, Tinga CJ, Wilson JHP, Westbroek DL. The significance of prostaglandin E2 in acute necrotizing pancreatitis in rats. Submitted.
26. Mehta J, Mehta P, Ostrowski N. Calcium blocker diltiazem inhibits platelet activation and stimulates vascular prostacyclin synthesis. Am J Med Sci 1986;291:20-24.
27. Farias LR, Frey CF, Holcroft JW, Gunther R. Effect of prostaglandin blockers on ascites fluid in pancreatitis. Surgery 1985;98:571-578.
28. Steer ML, Meldolesi J, Figarella C. Pancreatitis: the role of lysosomes. Dig Dis Sci 1984;29:934-938.
29. Gabryelewicz A, Dlugosz J, Brzozowski J, et al. Prostacyclin: effect on pancreatic lysosomes in acute experimental pancreatitis in dogs. Mt Sinai J Med 1983;50:218-224.
30. Lefer AM. The role of lysosomes in circulatory shock. Life Sci 1976;19:1803-1810.
31. Wereszczynska U, Dlugosz J, Gabryelewicz A, Andrzejewska A. The lung lysosomal hydrolases and phospholipases in acute experimental pancreatitis with reference to heparin treatment. Path Res Pract 1986;181:576-585.
32. Triebling AT, Dlugosz J, Brzozowski J, et al. The renal lysosomes in acute experimental pancreatitis in dogs treated with prostacyclin (PGI2). Path Res Pract 1984;178:280-288.
33. Dlugosz J, Gabryelewicz A, Andrzejewska A, et al. Prostacyclin (PGI2) stabilizes hepatic lysosomes during acute experimental pancreatitis in dogs. Z Exp Chir 1982;15:210-218.

CHAPTER 10

THE EFFECTS OF A LONG-ACTING SOMATOSTATIN ANALOGUE (SMS 201-995) ON EICOSANOID SYNTHESIS AND SURVIVAL IN RATS WITH ACUTE NECROTIZING PANCREATITIS.

Bart van Ooijen (1), Wil J Kort (1), Cor J Tinga (2), Steven WJ Lamberts (3), JH Paul Wilson (3), Dick L Westbroek (1).

Departments of Experimental Surgery (1) and Internal Medicine (3), Faculty of Medicine, Erasmus University, Rotterdam; Department of Pathology (2), Bronovo Hospital, The Hague.

10.1. ABSTRACT

The effects of a long-acting somatostatin analogue (SMS 201-995) were studied in a rat model of acute necrotizing pancreatitis (ANP). ANP was induced by retrograde injection of 5% sodium taurocholate (0.1 ml/100 g body weight) into the biliopancreatic duct.

The ANP induced in this model caused 100% mortality within 24 hours. SMS treatment decreased mortality to 40% ($p < 0.05$) when started before the induction of ANP. When treatment was delayed mortality increased to 75%.

After induction of ANP the serum amylase and lipase levels increased significantly ($p < 0.001$). In fasting animals the serum insulin levels and the glucose level in whole blood dropped significantly ($p < 0.001$). Administration of SMS 201-995 did not influence the increase in the serum concentrations of amylase, but the increase in serum lipase levels was significantly lowered ($p < 0.05$). The low levels of serum insulin and the glucose level in whole blood were not influenced by SMS administration.

In SMS-treated animals the volume of ascitic fluid was less than in controls ($p < 0.05$ and 0.01). Moreover less peritoneal fat necrosis was seen, suggesting a reduction in toxic factors in the ascitic fluid.

SMS treatment caused a sixfold increase in the levels of circulating

6-keto-PGF1alpha, the product of prostaglandin I2 synthesis ($p < 0.01$), while the TXB2 levels, reflecting thromboxane A2 activity, increased by 177%. It is suggested that SMS 201-995 is the activator of prostaglandin I2, thereby influencing the important TXA2/PGI2 ratio.

10.2. INTRODUCTION

The exact pathogenesis of acute necrotizing pancreatitis (ANP) is not clear, despite extensive investigation. As long ago as 1896 Chiari suggested that the main cause of pancreatitis was autodigestion of the pancreas by pancreatic enzymes. The activated enzymes would also enter the systemic circulation, thereby inducing multi-organ failure. However, inhibitors of both activated pancreatic enzymes (e.g. aprotinin) (1,2) and exocrine pancreatic secretion (calcitonin, glucagon) (3,4) have been used for the treatment of AP with little success.

Somatostatin (SRIF) is also known to be an inhibitor of pancreatic secretion (5). Moreover, SRIF is reported to have a cytoprotective effect on the gastrointestinal tract (6). Some studies suggest a beneficial role for SRIF in the treatment of AP (7,8,9); this is possibly due to its cytoprotective action rather than the inhibition of pancreatic secretion. Prostaglandins also have cytoprotective properties, especially prostacyclin (PGI2) and prostaglandin E (PGE) (10-13). The thromboxane A2 (TXA2)/PGI2 ratio appears to have broad biological significance to cell regulation (10,14). We have previously shown that the balance between these eicosanoids is disturbed in experimental ANP (15), and that restoration of this balance improves the survival rate (16).

The present study was undertaken to determine whether a long-acting somatostatin analogue (SMS 201-995) has a beneficial effect in ANP and, if so, whether such an effect could be related to changes in eicosanoid levels.

10.3. MATERIALS AND METHODS

Inbred male Wag/Rij rats (200-250 g) were used. Acute pancreatitis was induced by retrograde injection of 5% sodium taurocholate (STC) (0.1 ml/100 g body weight) into the biliopancreatic duct, as previously reported (15).

SMS 201-995 (SMS), a gift from Sandoz, Uden, the Netherlands, was

administered by a subcutaneous injection in a dose of 2 mu-gram/kg. Dilutions in 0.9% NaCl (1 mu-gram/ml) were prepared on the day of injection.

Nineteen animals received SMS 15 minutes before the induction of ANP, and 6 and 12 hours afterwards. In group 1 (12 animals) 0.5 ml of blood were collected before SMS treatment (zero time) and 12 and 24 hours after the induction of ANP. This blood was used to assess the serum amylase and lipase levels. In group 2 (7 animals) 1.0 ml of blood was collected at zero time, and 3, 6 and 12 hours after induction of ANP. This blood was used for determination of the plasma TXB2, 6-keto-PGF1alpha and PGE2 levels.

A control group (group 3, 7 rats) received a subcutaneous injection of 0.9% NaCl instead of SMS. In this control group 0.5 ml of blood were collected at 0, 12 and 24 hours for assessment of the serum amylase and lipase levels.

The 26 animals of groups 1, 2 and 3 were allowed to eat and drink ad libitum.

Another group of 12 animals (group 4) received the first SMS injection 30 minutes after the induction of ANP. The second and third doses were given 6 and 12 hours, respectively, after induction. Control animals (group 5, 8 rats) received an equal volume of 0.9% NaCl instead of SMS. These two groups fasted during a test period of 24 hours. Blood (1.0 ml) was collected at zero time and 12 and 24 hours after induction of ANP for determination of the amylase, lipase and insulin levels in serum. Whole blood glucose levels were also measured.

All blood samples were taken from the orbital venous plexus via a heparinized capillary tube. The blood samples for the insulin, amylase and lipase determinations were immediately centrifuged and the serum was stored at -30 degrees C until assay. The blood samples for the prostaglandin determinations were collected in tubes containing 20 mu-liter of sodium heparin (5000 U/ml Thromboliquine (R)) and 50 mu-liter of indomethacin (0.1 mg/ml in 0.1 M phosphate buffer, pH 8); 0.9% NaCl was added to obtain a volume of 1.5 ml. The blood samples were centrifuged immediately at 1400 x g for 10 minutes and the plasma stored at -70 degrees C until assay.

All blood taken was replaced by an equal volume of saline administered subcutaneously.

Survival time for the rats in groups 1, 3, 4 and 5 was noted and survivors were sacrificed after 36 hours. The rats in group 2 were sacrificed after the last blood samples were collected at twelve hours. The ascitic fluid in the

abdomen was collected and measured. In all cases the pancreas was removed, fixed in 4% formalin and embedded in paraffin. Sections were assessed by light microscopy. The amount of acinar cell necrosis was expressed as a percentage of the whole gland on the basis of a double blind microscopic evaluation of the slides. Differences in histology between test and control groups were tested with Fischer's test for 2 x 2 tables.

Analytical methods:

Amylase activity was determined using the enzymatic color test of Boehringer Mannheim (Automatenpackung Boehringer Mannheim alpha-amylase DHP, no. 568 589) with p-nitrophenyl-alpha,D-malto-heptaosid as substrate. The activity of amylase was expressed in units/l.

Lipase activity was determined using the UV-test of Boehringer Mannheim (Automatenpackung Boehringer Mannheim lipase, triacylglycerol lipase E.C. 3.1.1.3. UV test, no. 262 358). This method has been described by Ziegenhorn et al. (17). The activity of lipase was expressed in units/l.

Blood glucose was determined using the Ames Gluco System TM in Glucometer (R) II (Ames Division, Miles Laborotories Ltd, Stoke Poges, Slough , England). The blood glucose level was expressed in mmol/l.

Serum levels of insulin were determined by insulin radioimmunoassay (Immuno nuclear (R), cat. no. 0600) supplied as a kit. The insulin levels in serum were expressed in milliunits/l (mU/l).

Prostaglandin determinations were done as described previously (15).

Statistical analysis of the amylase, lipase, eicosanoid and survival data was carried out using the Mann Whitney U test. The serum insulin and the blood glucose determinations and the amount of ascitic fluid were tested with the Student's t-test for paired and unpaired data. Results are given as mean ± standard error.

10.4. RESULTS

Acute necrotizing pancreatitis was effectively induced by injecting STC into the biliopancreatic duct, as was proven by histological examination and the finding of elevated serum levels of amylase and lipase. The serum amylase

and lipase levels found for the test and control groups at zero time were similar.

After induction of ANP serum levels of amylase increased significantly in both the test and control groups (1,3,4,5) with a maximum being reached at 12 hours. Rats receiving SMS 201-995 either 15 minutes before or 30 minutes after the induction of ANP did not have significantly different amylase levels from the controls (table 1).

Table 1: Changes in the serum levels of amylase in acute pancreatitis after treatment with SMS 201-995.

group	zero time	t = 12	t = 24
		*	*
SMS (-15) (group 1)	9133±1454 (12)	38666± 5444 (12)	37314±8971 (7)
		*	
controls (group 3)	9429± 425 (7)	42750± 3292 (5)	--
		*	
SMS (+30) (group 4)	9600± 425 (12)	27133± 3791 (6)	13300±2050 (2)
		*	
controls (group 5)	9650± 684 (8)	45057±14714 (7)	15000 (1)

Results are given in units/l and expressed as means ± SD. The number of animals is indicated in parentheses. * = $p < 0.01$ significant rise in comparison with baseline values (Mann Whitney U test).

Serum lipase concentrations also increased significantly in all groups, although the increase was significantly less in SMS 201-995 group 1 ($p < 0.05$). When SMS treatment was delayed until 30 minutes after the induction of ANP, serum levels of lipase were still lower than the control values, but the difference was not statistically significant (table 2).

Blood glucose concentrations decreased significantly 12 hours after induction of ANP. This was not influenced by treatment with SMS 201-995. The values returned to baseline values 24 hours after starting the experiment (table 3).

Serum insulin levels decreased significantly 12 hours after induction of

ANP; no difference was seen between the SMS group and control group. The serum levels returned to baseline values 24 hours after the start of the experiment (table 4).

Table 2: Changes in the serum levels of lipase in acute pancreatitis after treatment with SMS 201-995.

group	zero time	t = 12	t = 24
SMS (-15)(group 1)	0 (12)	2695± 452 (11) [a,b]	1399±541 (7)
controls (group 3)	0 (7)	7510±2906 (5) [a]	---
SMS (+30)(group 4)	0 (12)	2350± 475 (6) [a]	650±106 (2)
controls (group 5)	0 (8)	4284±1023 (7) [a]	800 (1)

Results are given in units/l and expressed as means ± SD. The number of animals is indicated in parentheses. a = $p < 0.01$ statistically significant in comparison with baseline values (Mann Whitney U test); b = $p < 0.05$ statistically significant in comparison with control values (Mann Whitney U test).

Table 3: Blood glucose levels in acute necrotizing pancreatitis.

group	zero time	t = 12	t = 24
controls (group 5)	5.7±0.2 (8)	3.2±0.5 (7) [a]	5.6 (1)
SMS (group 4)	5.9±0.3(12)	3.3±0.5 (6) [a]	4.5±0.5 (3)

Results are given in mmol/l and expressed as means ± SD. The number of animals is indicated in parentheses. a = statistically significant with respect to baseline values ($p < 0.001$, Student's t-test).

Table 4: Serum insulin levels in acute necrotizing pancreatitis.

group	zero time	t = 12	t = 24
controls (group 5)	18.0±2.5 (8)	4.3±0.8 (8) *	16.1 (1)
SMS (group 4)	18.9±2.5 (12)	5.1±0.6 (6) *	16.3±1.8 (3)

Results are given in mU/l and expressed as means ± SD. The number of animals is indicated in parentheses. * = $p < 0.001$ statistically significant with respect to baseline values (Student's t test).

EICOSANOID DETERMINATIONS

The use of SMS 201-995 for the treatment of ANP was associated with some effects on eicosanoid formation. TXB2 levels increased to almost three times the baseline value at three hours ($p < 0.01$) and then decreased. The levels of 6-keto-PGF1alpha increased to six times the baseline value at three hours ($p < 0.01$). PGE2 levels decreased in the first six hours, but 12 hours after induction of ANP the levels had returned to the baseline value. Details are given in table 5:

Table 5: Plasma TXB2, 6-keto-PGF1alpha and PGE2 levels in SMS 201-995-treated animals.

	zero	t=3	t=6	t=12
TXB2	100	277 *	180	173
6-keto-PGF1a	100	617 *	303	280
PGE2	100	33	49	93

Results are given as percentage change in the median baseline values. The median baseline values, which equal 100%, are given in pg/ml: TXB2 64 pg/ml (range 58-165), 6-keto-PGF1alpha 66 pg/ml (range ≤25-245), PGE2 75 pg/ml (range ≤25-165). * = $p < 0.01$ statistically significant with respect to baseline values (Mann Whitney U test).

PATHOLOGY AND SURVIVAL

After the induction of ANP the animals became severely ill. The animals treated with SMS 201-995 before the induction of ANP appeared to be in a somewhat better condition than the controls. All control animals in both control groups died within 24 hours. In SMS group 1 (treatment started before ANP) 60% survived for 36 hours ($p < 0.05$). Moreover, the animals of this group that did not survive still lived longer (mean 20.5 ± 2.5) than the controls (mean 13.1 ± 1.3) ($p < 0.05$). When SMS was given after the induction of ANP 25% of the animals still survived. A number of these animals died when the blood samples (1.0 ml) were drawn 12 hours after the induction of ANP. Results are summarized in table 6.

All animals dying of the disease developed ascites. The largest volumes had accumulated within 24 hours, while at 36 hours most ascites had disappeared. The amounts measured in the different groups are listed in table 7. Even the non-survivors of the two SMS groups developed less ascitic fluid than the controls ($p < 0.05$ and 0.01).

Within 24 hours some animals exhibited distension of the stomach and proximal bowel. A slight hydrothorax was sometimes encountered. No difference was seen between the SMS and control groups in this respect. Fat necrosis, encountered throughout the peritoneal cavity, increased with time. However, in the two SMS groups fat necrosis was very mild compared to the moderate to severe necrosis observed in the untreated control groups.

A few differences in histology were found between the control and test groups, usually due to the longer survival time for the test groups. A severe inflammatory response was seen only in those animals of the test groups with a prolonged survival time since the inflammatory response intensifies with time. As a rule, severe edema only occurred in surviving animals. Small hemorrhages developed in all groups. Dilatation of ducts was seen in all groups and in about 60% of the animals that died within 24 hours. Fibrinoid necrosis of small blood vessels was seen in 60% of the control animals and 20-30% of the test animals dying of the disease. The amount of acinar cell necrosis differed between the groups. All animals in the control groups developed more than 25% acinar cell necrosis. When SMS treatment was started before induction of ANP, 80% of the animals that died of the disease exhibited less than 25% necrosis

non-surviving animals showed less than 25% acinar cell necrosis. The longer the animals survived the greater the increase in acinar cell necrosis.

Table 6: Survival time in acute necrotizing pancreatitis.

group	survivors	non-survivors
controls (group 3)	0%	100% (13.1±1.3)
SMS (-15)(group 1)	60% *	40% (20.5±2.5) **
controls (group 5)	0%	100% (13.7±2.1)
SMS (+30)(group 4)	25%	75% (11.3±0.6)

The percentage survivors is given. The mean survival time for the non-survivors is given ± SD in parentheses.
* = $p < 0.05$ statistically significant difference with respect to control group (Fischer's test for 2x2 tables).
** = $p < 0.01$ statistically significant difference with respect to control group (Mann Whitney U test).

Table 7: The amount of ascitic fluid formed in acute necrotizing pancreatitis in rats.

group	amount in ml
controls (group 3) (7)	6.9 ± 1.8
SMS (-15) (group 1) (5)	5.4 ± 1.8 *
controls (group 5) (8)	6.8 ± 1.2
SMS (+30) (group 4) (9)	4.9 ± 1.1 **

Results are given in ml ± SD. The number of animals is indicated in parentheses (non-surviving animals). For surviving animals the amount of ascitic fluid collected was usually less than 1.0 ml. *,** = statistically significant with respect to control group (* = $p < 0.05$, ** = $p < 0.01$, Student's t-test).

10.5. DISCUSSION

This study demonstrates the effects of SMS 201-995 (SMS)in acute necrotizing pancreatitis in rats. Depending on the time of administration SMS obviously influenced the serum lipase levels, the accumulation of ascitic fluid, the amount of fat necrosis in the peritoneal cavity and the survival time.

When SMS treatment was started before the induction of ANP, a significant improvement in the percentage survivors was seen (from zero to 60%) ($p < 0.05$). Moreover, the survival time for non-survivors was significantly longer (20.5 hr) than for control animals (13.1 hr) ($p < 0.05$). Does SMS 201-995 prevent the onset of ANP? Our results do not point in that direction since serum amylase levels increased equally in the SMS and the control groups. Moreover the serum lipase levels also increased significantly, albeit less than in the control group; this might indicate a less aggressive form of ANP.

Differences were seen between the control and SMS groups in the amount of acinar cell necrosis, the volume of ascitic fluid and the severity of peritoneal fat necrosis. The reduction in acinar cell necrosis may be ascribed to a cytoprotective effect, as has been reported previously (6). The milder peritoneal fat necrosis in SMS-treated animals suggests that the ascitic fluid is not as toxic in these rats as in control animals. As a consequence resorption of ascitic fluid will cause fewer systemic effects in SMS-treated animals.

No differences between the two SMS groups could be found in the volume of ascitic fluid or the severity of fat necrosis. When SMS treatment was delayed, mortality increased and only 25% of the animals survived. Partly, this might be explained by the fact that in this group more blood had to be collected. It is also possible that our model is too aggressive, because pancreatic lesions are immediate and severe when a 5% sodium taurocholate solution is used (18,19). Perhaps there is yet another factor by means of which SMS, when administered early in the course of the disease, improves survival.

The lack of significant effects on survival in established pancreatitis contradicts the results of a study by Baxter et al. (9), who used a different model. In bile-induced AP in dogs somatostatin appeared to be effective but not when treatment was delayed 4 hours (8). No influence at all was observed in a rat study by Lankish et al. (20) and a clinical study of established

pancreatitis (21). Thus, the efficacy of somatostatin (SRIF) in AP has been questioned. Our study strengthens the opinion that SRIF is beneficial, especially when treatment is started early.

The influence of SRIF on ANP may be ascribed to its inhibitory effects on bile and pancreatic exocrine secretion (22-25). Inhibition of secretion is probably (at least in part) the result of the inhibition of GI hormones such as glucagon (22). However, substances that inhibit pancreatic secretion (calcitonin, glucagon) have proved to be of little value for the treatment of AP (3,4). Moreover, the serum levels of lipase and amylase do not point to a substantial role for the inhibitory activity of SRIF.

SRIF also has a marked vascular effect in the splanchnic area. When administered intravenously SRIF decreases all splanchnic blood flow, except for that in the hepatic artery (23,26,27). It usually does not influence systemic circulation (23,27). The decrease in splanchnic flow only, a transient phenomenon (24), can probably be explained by an inhibition of secretory activity, reflecting decreased circulatory demands (24,27). Inflammatory diseases usually increase the circulatory demand, whereas in ANP the flow is diminished (28,29). Information on the effect of SRIF on the circulation in the pancreas in patients with ANP is not available. However, SRIF administration helps to prevent shock (7,30), a condition that may facilitate the development of ANP (31).

An interesting feature of our study is the pronounced elevation of 6-keto-PGF1alpha levels which reflects prostacyclin production. Prostacyclin (PGI2) inhibits pancreatic exocrine secretion (32). As previously reported TXB2 levels (reflecting thromboxane A2 activity) are markedly elevated in ANP (a rise of 300%) and those of 6-keto-PGF1alpha just moderately (a rise of 60%) (15,33). The TXA2/PGI2 ratio is important in vascular homeostasis (especially of the microcirculation) and may also have a broader biological significance (10,14). Prostacyclin is a potent vasodilator and powerful inhibitor of platelet activation (10), while TXA2 is a potent vasoconstrictor and stimulus for platelet aggregation (14). In ANP vasoconstriction, poor vascular filling and spastic changes in the lobular vessels are seen (34). Moreover, ischemia is said to be a critical factor in the progression from edema to necrosis (31). In SMS 201-995-treated animals the TXA2/PGI2 ratio is changed in favor of PGI2. Influencing the ratio in this manner improved survival significantly, as reported in a previous study (16). Moreover, in SMS-treated animals the

plasma PGE2 levels, that are severely depressed in ANP (15), returned to baseline values within twelve hours. In ANP PGE2 activity is still significantly decreased 12 hours after induction (15). SRIF has been suggested to have direct cytoprotective properties (6,7), but prostacyclin is also presumed to have cytoprotective properties (10,14). In ANP prostacyclin stabilizes the lysosomal membranes (35). It appears likely that SMS 201-995 exerts its effect in ANP via the prostaglandin system, especially by influencing the TXA2/PGI2 ratio!

SMS 201-995 did not have a significant effect on the endocrine function of the pancreas in ANP. SRIF is said to reduce insulin levels (7,23,24) but ANP in itself causes a significant drop in the serum levels of insulin. In addition, SRIF is known to decrease (fasting) blood glucose levels in dogs (23), but in ANP glucose levels are significantly reduced in fasting rats even without SMS treatment and even in the presence of low insulin levels. The mechanism by which ANP causes a drop in insulin levels is not clear.

In conclusion SMS 201-995 has a beneficial effect on the course of ANP. When administered early survival time improves significantly. It seems likely that SMS influences ANP not by inhibition of the secretory activity, but rather by its cytoprotective effects. Somatostatin could therefore be the activator of prostaglandin I2, thereby influencing the important TXA2/PGI2 ratio!

10.6. REFERENCES

1. MRC Multicenter trial. Death from acute pancreatitis. Lancet 1977;2:632-635.

2. Imrie CW, Benjamin IS, Ferguson J, et al. A single center double blind trial of Trasylol therapy in primary acute pancreatitis. Br J Surg 1978;65:337-341.

3. Durr GHK. Therapeutic trials in acute pancreatitis. German experience. Gastroenterologie Clinique et Biologique 1978;11:954.

4. Imrie CW, Blumgart LH. Glucagon therapy in acute pancreatitis. Br Med J 1974;1:38.

5. Dollinger HC, Raptis S, Pfeiffer EF. Effects of somatostatin on exocrine and endocrine pancreatic function stimulated by intestinal hormones in man. Horm Metab Res 1976;8:74-78.

6. Szabo S, Usadel KH. Cytoprotection - organoprotection by somatostatin; gastric and hepatic lesions. Experientia 1982;38:254-256.

7. Schwedes U, Althoff PH, Klempa I, et al. Effect of somatostatin on bile-induced acute hemorrhagic pancreatitis in the dog. Horm Metab Res 1979;11:655-661.

8. Limberg B, Kommerell B. Treatment of acute pancreatitis with somatostatin. New Engl J Med 1980;303:284.

9. Baxter JN, Jenkins SA, Day DW, et al. Effects of somatostatin and a long-acting somatostatin analogue on the prevention and treatment of experimentally induced acute pancreatitis in the rat. Br J Surg 1985;71:382-385.

10. Moncada S, Vane JR. Prostacyclin and its clinical applications. Ann Clin Res 1984;16:241-252.

11. Gabryelewicz A, Dlugosz J, Brzozowski J, et al. Prostacyclin: effect on pancreatic lysosomes in acute experimental pancreatitis in dogs. Mt Sinai J Med 1983;50:218-224.

12. Miller P, Fischer N, Damann HG, Kather H, Simon B. The cytoprotective effect of 16,16-dimethyl prostaglandin E2 on bile salt induced damage to the human stomach. Br J Clin Pharmacol 1981;12:253-254.

13. Reber HA, Tweedie JH, Mosely JG. The cytoprotective effect of 16,16-dimethyl prostaglandin E2 on bile salt induced damage to the pancreas. Gastroenterology 1980;78:1241.

14. Bunting S, Moncada S, Vane JR. The prostacylin-thromboxane A2 balance: pathophysiological and therapeutic implications. Br Med Bull 1983;39:271-276.

15. Van Ooijen B, Kort WJ, Zijlstra FJ, Vincent JE, Wilson JHP, Westbroek DL. Prostanoid imbalance in experimental acute necrotizing pancreatitis in rats. Scand J Gastroenterol, accepted.

16. Van Ooijen B, Kort WJ, Tinga CJ, Wilson JHP, Westbroek DL. The significance of the thromboxane A2/prostaglandin I2 balance in acute necrotizing pancreatitis in rats. Submitted.

17. Ziegenhorn J, Neumann KW, Zwez W. Determination of serum lipase. Clin Chem 1979;25:1067.

18. Aho HJ, Koskensalo SM-L, Nevalainen TJ. Experimental pancreatitis in the rat. Sodium taurocholate-induced acute haemorrhagic pancreatitis. Scand J Gastroent 1980;15:411-416

19. Aho HJ, Nevalainen TJ. Experimental pancreatitis in the rat. Scand J Gastroent 1980;15:417-424.

20. Lankisch PG, Koop H, Winckler K, Folsch UR, Creutzfeldt W. Somatostatin therapy of acute experimental pancreatitis. Gut 1977;18:713-716.

21. Usadel KH, Uberla KK, Leuschner U. Treatment of acute pancreatitis with somatostatin: results of the multicenter double-blind trial (APTS-study). Dig

Dis Sci 1985;30:992.
22. Hanks JB, Kortz WJ, Andersen DK, Scott Jones R. Somatostatin suppression of canine fasting bile secretion. Gastroenterology 1983;84:130-137.
23. Price BA, Jaffe BM, Zinner MJ. Effect of exogenous somatostatin infusion on gastrointestinal blood flow and hormones in the conscious dog. Gastroenterology 1985;88:80-85.
24. Konturek SJ, Tasler J, Jaworek J, et al. Gastrointestinal secretory, motor, circulatory and metabolic effects of prosomatostatin. Proc Natl Acad Sci USA 1981;78:1967-1971.
25. Folsch UR, Lankisch PG, Creutzfeldt W. Effect of somatostatin on basal and stimulated pancreatic secretion in the rat. Digestion 1978;17:194-203.
26. Thulin L, Samnegard H. Circulatory effects of gastrointestinal hormones and related peptides. Acta Chir Scand 1978;482(S):73-74.
27. Samnegard H, Thulin L, Andreen M, et al. Circulatory effects of somatostatin in anaesthetized dogs. Acta Chir Scand 1979;145:209-212.
28. Goodhead B. Acute pancreatitis and pancreatic bloodflow. Surg Gyn Obstet 1969;69:331-340.
29. Donaldson LA. Pancreatic circulation reduces rapidly in acute pancreatitis. Surgery 1978;84:313-321.
30. Schwedes U, Wdowinski J, Althoff PH, Usadel KH. Somatostatin mediated decrease of shock equivalents in various animal experiments. Acta Endocrinol (Suppl) 1980;234:142.
31. Warshaw AL, O'Hara PJ. Susceptibility of the pancreas to ischemic injury in shock. Ann Surg 1978;188:197-201.
32. Konturek SJ, Tasler J, Jaworek J, Cieszkowski M, Pawlik W. Prostacyclin inhibits pancreatic secretion. Am J Physiol 1980;238:(G)531-536.
33. Van Ooijen B, Ouwendijk RJT, Kort WJ, et al. Increased thromboxane B2 levels in acute necrotizing pancreatitis in rats. The effects of flunarizine, dazoxiben and indomethacin. Scand J Gastroenterol, accepted.
34. Schiller WR, Anderson MC. Microcirculation of the normal and inflamed canine pancreas. Ann Surg 1975;181:466-470.
35. Gabryelewicz A, Dlugosz J, Brzozowski, et al. Prostacyclin: effect on pancreatic lysosomes in acute experimental pancreatitis in dogs. Mt Sinai J Med 1983;50:218-224.

CHAPTER 11

DISCUSSION AND CONCLUSIONS.

Acute necrotizing pancreatitis is a disease with a considerable morbidity and mortality. Unfortunately, the pathogenesis and pathophysiology are not clearly understood, which means that causal treatment is not possible. An effective approach to the management of acute necrotizing pancreatitis has not yet been discovered. The progress that has been made in the treatment of human acute necrotizing pancreatitis is purely a case of improved intensive care techniques, including better ventilation support, improved monitoring of cardiac function and the possibility of hemofiltration. Therefore a better understanding of the pathogenesis and pathophysiology of acute necrotizing pancreatitis will create the possibilities of finding effective causal treatment modalities.

This thesis was planned with this problem in mind. In chapter 1 the aims of the study are formulated. Prostaglandins and thromboxanes are potent mediators that play a role in several diseases. It was thought that prostaglandins and thromboxanes may mediate many of the local disorders in acute necrotizing pancreatitis as well as the systemic manifestations of the pancreatic inflammation. Unfortunately very few studies in this research field have yet been published and no critical comparison can be made in this chapter.

To unravel the possible role of eicosanoids in acute necrotizing pancreatitis we measured plasma levels of thromboxane B2, the stable metabolite of thromboxane A2, in venous and arterial blood in a rat model of acute necrotizing pancreatitis. We had to turn to an experimental model of acute necrotizing pancreatitis because the number of patients with acute necrotizing pancreatitis admitted to the hospital is limited and the diagnosis is not easily made; moreover the degree of inflammation may vary considerably. In the model described in chapter 5 these problems are not present.

Thromboxane B2 levels rose sharply and significantly when measured 3, 6 and 12 hours after the onset of acute necrotizing pancreatitis with a peak (300% increase) at 6 hours (chapters 6,7). Thromboxane A2 is thought to mediate ischemia, because it is a strong vasoconstrictor and platelet aggregator. Ischemia may be the important event that determines the progression of edematous pancreatitis to necrotizing pancreatitis. Thromboxane A2 is also

thought to act as a cytolytic agent.

We also measured the plasma levels of 6-keto-PGF1alpha (the stable metabolite of prostacyclin) and prostaglandin E2 in arterial blood from rats with acute necrotizing pancreatitis. The levels of 6-keto-PGF1alpha rose slightly but significantly when measured 3, 6 and 12 hours after the onset of acute necrotizing pancreatitis with a small peak (68% increase) at 6 hours. The levels of prostaglandin E2 decreased steadily from 100% (baseline values) to 36% 12 hours after the induction of acute necrotizing pancreatitis (chapter 7).

Prostaglandins I2 and E2 are dilators of the mesenteric vascular bed, thereby increasing microcirculatory blood flow in intestinal organs. They also have cytoprotective properties. Prostaglandin E2 reportedly protects the mucosa in the pancreatic duct against noxious agents that cause inflammation and necrosis. Prostaglandin I2 is known to stabilize lysosomes of renal, pulmonary and hepatic origin in acute pancreatitis. With respect to the pancreas prostaglandin I2 exhibits a cytoprotective effect on the pancreatic lysosomes in less advanced acute pancreatitis in dogs. Cytoprotection may be important for maintenance of the integrity of both acinar cells and cell organelles. These actions may possibly prevent the release of activated digestive enzymes. Prostaglandins I2 and E2 are both inhibitors of pancreatic secretion. In the event of inflammation pancreatic secretion is thought to facilitate progression of the disease.

Elevated levels of prostaglandins and thromboxanes are due to enhanced synthesis or decreased clearance from the circulation. Eicosanoids are not stored in tissues and biosynthesis must immediately precede release. Inactivation occurs in lungs, liver, spleen, kidneys and blood vessels (chapter 4). It is possible, but unlikely, that the clearance of some compounds (i.e. prostaglandin I2 and thromboxane A2) is decreased while the clearance of others (i.e. prostaglandin E2) is enhanced. The measured elevation of 6-keto-PGF1alpha and thromboxane B2 levels after the induction of acute necrotizing pancreatitis is thus most probably due to an enhanced synthesis, whereas the decreasing prostaglandin E2 levels most probably reflect a depressed synthesis.

An important question that remains to be answered is whether the changes in the levels of certain eicosanoids in experimental acute necrotizing pancreatitis have any significance or are they merely epiphenomena. In an

attempt to answer this question we inhibited prostaglandin and thromboxane compounds at the level of synthesis and measured the effects of this inhibition on survival time.

Inhibition of the enzyme phospholipase A2 by chloroquine increased the survival rate by 30% (chapter 7). Phospholipase A2 is thought to be the main enzyme responsible for eicosanoid synthesis and phospholipase A2 blockers inhibit prostaglandin and thromboxane synthesis as well as synthesis of the leukotrienes.

However, although the expected rise in thromboxane B2 levels was effectively inhibited, the levels of 6-keto-PGF1alpha and prostaglandin E2 remained slightly elevated 3 and 6 hours after the onset of the disease. It is possible that these prostaglandins are formed via pathways other than by phospholipase A2. On the other hand the concentration of active chloroquine may not have been high enough to block prostaglandin synthesis in the first 6 hours of the disease.

Flunarizine, a calcium entry blocker, is also thought to act as a phospholipase A2 inhibitor. Flunarizine pretreatment in experimental acute necrotizing pancreatitis decreased the mortality by 30% (chapter 6). Flunarizine does not interfere with the biosynthesis of thromboxane A2 in thrombin-stimulated thromboxane A2 release; however in acute necrotizing pancreatitis endogenous thromboxane A2 activity decreased after flunarizine pretreatment, pointing to central inhibition of thromboxane A2 synthesis by inhibition of phospholipase A2. Phospholipase A2 is indeed highly dependent upon calcium. We did not know, however, that calcium entry blockers are able to stimulate vascular prostacyclin synthesis, again possibly via pathways other than by phospholipase A2.

Although chloroquine and flunarizine pretreatment improved survival slightly, no real conclusion can be drawn about the optimal level of inhibition of phospholipase A2 because effective inhibition of thromboxane A2 synthesis is accompanied by an increase in 6-keto-PGF1alpha levels.

Whether the leukotriene pathway is effectively inhibited by chloroquine and flunarizine was not checked, but inhibition of leukotriene synthesis by FPL 55712 did not improve survival significantly. In view of these findings the leukotriene pathway is not thought to contribute substantially to mortality in experimental acute necrotizing pancreatitis (chapter 7).

Indomethacin is an accepted and well-documented compound that inhibits the

cyclooxygenase enzyme system. Pretreatment with indomethacin is associated with a high mortality rate for rats with experimental acute necrotizing pancreatitis. In fact the histological alterations were worse after pretreatment with indomethacin (chapter 6). In accordance with other studies this supports the hypothesis that complete inhibition of prostaglandin and/or thromboxane synthesis causes exacerbation of the acute necrotizing pancreatitis.

As certain eicosanoids may be important in pancreatic disease a question remaining to be answered is which eicosanoids play a role in acute necrotizing pancreatitis. On the basis of the known properties of the compound we hypothesized a detrimental role for an excess of thromboxane A2. Although we tentatively suggest that some of the complications of acute necrotizing pancreatitis may be mediated by thromboxane A2, experience with indomethacin indicates that some of the other cyclooxygenase products have a beneficial function and may play a role in the basic homeostasis. According to our hypothesis a shortage of prostaglandin E2 and a relative shortage of prostaglandin I2 are detrimental in experimental acute pancreatitis.

Specific inhibition of thromboxane A2 synthesis by dazoxiben decreased the mortality rate by 40% (chapter 6). Administration of prostaglandin E2 also decreased the mortality rate by 40% (chapter 8). Administration of iloprost (a stable analogue of prostaglandin I2) decreased mortality by 50% (chapter 9). These findings indicate that the hypothetical roles of thromboxane A2, prostaglandin E2 and prostaglandin I2 are probably true. However, none of the differences in survival time exhibit statistical significance.

A statistically significant difference in mortality rate was found for the simultaneous administration of a thromboxane A2 inhibitor and prostaglandin E2 or iloprost (a stable derivative of prostaglandin I2). After pretreatment with dazmegrel (a specific inhibitor of thromboxane A2 synthesis) and iloprost 90% of the animals survived for more than 48 hours ($p < 0.01$) (chapter 9). Dazmegrel probably also augments endogenous prostacyclin synthesis. The effects on survival were less pronounced when dazmegrel was administered together with prostaglandin E2; 60% of the animals survived for 36 hours ($p < 0.05$) (chapter 8). When flunarizine was combined with either prostaglandin E2 or iloprost 60% of the animals survived for 72 hours ($p < 0.05$) (chapters 8,9).

The conclusion is justified that, in particular, the thromboxane A2/prostacyclin ratio is important. In experimental acute necrotizing

pancreatitis the balance between these eicosanoids is disturbed and a change in this ratio improved the survival rate.

The mechanism of action of the thromboxane A2/prostacyclin system in experimental acute necrotizing pancreatitis is not fully clear. The amount of ascitic fluid was significantly reduced in animals treated with either dazmegrel/iloprost or dazmegrel/prostaglandin E2. Both prostaglandins I2 and E2 cause an increase in microvascular permeability. The lower volume of ascitic fluid is possibly a reflection of the effects of thromboxane A2/prostacyclin and thromboxane A2/prostaglandin E2 on the pancreas. Prostaglandins I2 and E2 may act at the cellular level by protecting against cellular degeneration. This is also supported by the fact that less necrosis was found in dazmegrel/iloprost and dazmegrel/prostaglandin E2-treated animals.

The post mortem and histological results suggest that local factors within the pancreatic region are not exclusively important to survival. For example, even surviving animals treated with dazmegrel and iloprost exhibited considerable pancreatic necrosis. A beneficial role of the thromboxane A2/prostacyclin system on other organs is likely. Prostaglandin I2 is known to stabilize hepatic, renal and lung lysosomes in experimental acute necrotizing pancreatitis. These organs are frequently involved during the course of acute necrotizing pancreatitis. The thromboxane A2/prostacyclin system is also important in endotoxic shock and for renal ischemia (chapter 4).

In chapter 10 the effects of somatostatin on experimental acute necrotizing pancreatitis were evaluated. Somatostatin is a known inhibitor of pancreatic secretion, like prostaglandins E2 and I2, and is also reported to have cytoprotective properties. Moreover a beneficial influence on the course of the disease has been demonstrated in some cases of experimental and clinical acute pancreatitis. It was thought that somatostatin works via the prostaglandin system. When SMS 201-995 (a long-acting somatostatin analogue) was given before acute necrotizing pancreatitis was induced, survival time improved significantly. In addition, the plasma 6-keto-PGF1alpha levels were significantly elevated, whereas the increase in thromboxane B2 was less pronounced. In view of the similarities it is suggested that prostaglandin I2 is the ultimate mediator of somatostatin. Because somatostatin is a harmless hormone, that can be administered easily, this finding opens an interesting field for clinical research.

This thesis indicates that certain eicosanoids influence the course of

experimental acute necrotizing pancreatitis. On the basis of these findings the next step would seem to be to determine whether eicosanoid imbalance occurs in human pancreatitis. An eicosanoid imbalance opens interesting perspectives for further research. The balance between thromboxane A2 and prostaglandin I2 can be restored and improved by inhibition of thromboxane synthesis in combination with stimulation of prostacyclin by SMS 201-995 or administration of iloprost.

SUMMARY.

The role of certain eicosanoids (prostaglandins E2 and I2 and thromboxane A2) in acute necrotizing pancreatitis in rats was investigated in this thesis. With respect to acute pancreatic inflammation it is hypothesized that these eicosanoids exert a local influence on the pancreas and also mediate many of the systemic complications in acute pancreatitis, including severe shock, respiratory insufficiency, renal failure and hepatic failure.

In chapter 1 the aim of the study is formulated. In chapter 2 the etiological factors responsible for the development of human acute pancreatitis are described and the possible pathogenetic mechanisms are discussed briefly. In chapter 3 the known aspects of the pathogenesis and pathophysiology of human acute pancreatitis are considered in more detail, with special attention to the pancreatic enzymes. The possible role of eicosanoids in the pathogenesis and pathophysiology is discussed briefly. In chapter 4 the influence of prostaglandins and thromboxane A2 on normal pancreatic physiology and pancreatic disease is described.

A model of acute necrotizing pancreatitis in rats is described in chapter 5. This model is comparable, in particular, to the acute pancreatitis that is associated with biliary tract disease in man.

In chapter 6 we measured thromboxane B2 levels in plasma after induction of acute pancreatitis in rats. The levels of thromboxane B2, the stable end-product of thromboxane A2, increased significantly. Inhibition of thromboxane A2 synthesis by indomethacin, dazoxiben or flunarizine did not dramatically alter survival time in acute pancreatitis. An exclusive role for thromboxane A2 could thus not be shown; a potential role for other arachidonate metabolites could, however, not be ruled out by these results.

In chapter 7 we measured thromboxane B2 levels in plasma in relation to the levels of 6-keto-PGF1alpha (the stable product of prostacyclin) and prostaglandin E2. The significant increase in thromboxane B2 levels was accompanied by a slight increase in 6-keto-PGF1alpha levels, whereas the prostaglandin E2 levels decreased. We hypothesized a detrimental effect caused by an excess of thromboxane A2, a shortage of prostaglandin E2 and a relative shortage of prostacyclin.

In chapter 8 the effects of the administration of prostaglandin E2 on the mortality rate for rats are described. The mortality rate decreased by 40%

when prostaglandin E2 was given. When thromboxane A2 was inhibited simultaneously the mortality rate decreased further (by 60%, $p < 0.05$).

In chapter 9 the effects of the administration of iloprost (a stable analogue of prostacyclin) were tested. Iloprost therapy decreased the mortality rate by 50%. A further decrease in the mortality rate was achieved when thromboxane A2 was inhibited simultaneously. When flunarizine (a calcium entry blocker that inhibits thromboxane A2) was given 60% survived, whereas dazmegrel (a thromboxane A2 synthetase inhibitor) resulted in a 90% survival rate at 48 hours ($p < 0.01$).

In chapter 10 the effects of a long-acting somatostatin analogue (SMS 201-995) were studied. When administered early in the course of acute necrotizing pancreatitis, SMS 201-995 improved survival time significantly. SMS 201-995, a harmless substance, caused a sixfold increase in the circulating levels of 6-keto-PGF1alpha, whereas thromboxane B2 levels increased by only 177%. It is suggested that prostacyclin is the ultimate mediator of somatostatin, thereby influencing the important thromboxane A2/prostaglandin I2 ratio.

In chapter 11 all results are discussed. This thesis indicates that prostaglandins I2 and E2 and thromboxane A2 influence the course of experimental acute necrotizing pancreatitis. The balance between thromboxane A2 and prostacyclin appears to be especially important. In view of these findings the next step would seem to be to determine whether an eicosanoid imbalance exists in human acute pancreatitis. An eicosanoid imbalance opens interesting perspectives for further research. The balance between thromboxane A2 and prostaglandin I2 can be restored and improved by inhibition of thromboxane synthesis in combination with stimulation of prostaglandin I2 by SMS 201-995 or administration of iloprost.

SAMENVATTING.

In dit proefschrift wordt een onderzoek naar de mogelijke rol van bepaalde eicosanoiden (prostaglandinen E2 en I2, thromboxaan A2) in acute necrotizerende pancreatitis in ratten beschreven. Eicosanoiden zijn biologisch actieve mediatoren, die een rol spelen in de pathogenese en pathofysiologie van verschillende ernstige ziektebeelden. De hypothese met betrekking tot acute necrotizerende ontsteking van het pancreas is dat eicosanoiden lokaal in het pancreas werkzaam zijn en tevens mediatoren zijn van de systemische complicaties van acute pancreatitis zoals ernstige shock, longinsufficientie, nierinsufficientie en het levercelverval.

In hoofdstuk 1 wordt de doelstelling van het onderzoek geformuleerd. In hoofdstuk 2 worden de verschillende factoren, die verantwoordelijk worden geacht voor het ontstaan van de ziekte in mensen, beschreven en de mogelijke pathogenetische mechanismen kort aangestipt. In hoofdstuk 3 worden de bekende literatuurgegevens over de pathogenese van deze ernstige ziekte verder uitgediept. De rol, die de verschillende pancreasenzymen hierin spelen wordt beschreven. De mogelijke rol van de eicosanoiden wordt kort aangestipt. In hoofdstuk 4 wordt de rol die prostaglandinen en thromboxaan A2 in de fysiologie van het pancreas spelen, beschreven in normale en afwijkende situaties.

Een model voor acute necrotizerende pancreatitis in ratten wordt beschreven in hoofdstuk 5. Dit model is met name vergelijkbaar met de acute necrotizerende pancreatitis die ontstaat in samenhang met galsteenlijden. In hoofdstuk 6 worden metingen gedaan naar de plasma spiegels van thromboxaan B2 na de inductie van acute pancreatitis. De plasma spiegels van thromboxaan B2 (het stabiele eindprodukt van thromboxaan A2) stijgen significant. Remming van de thromboxaan A2 synthese door indomethacine, dazoxiben en flunarizine verbetert de overleving van de ratten met acute pancreatitis echter niet significant. Hoewel een voorname rol voor thromboxaan A2 dus niet kon worden aangetoond, kon een mogelijke rol van andere arachidonzuurmetabolieten niet worden uitgesloten. Daarom werden in hoofdstuk 7 de plasma spiegels van thromboxaan B2 gemeten in relatie tot die van 6-keto-PGF1alpha (het stabiele produkt van prostaglandine I2) en prostaglandine E2. De significante verhoging van thromboxaan B2 spiegels blijkt gepaard te gaan met een lichte verhoging van 6-keto-PGF1alpha spiegels en sterk verlaagde spiegels van prostaglandine

E2. De hypothese wordt nu dat in acute pancreatitis een ongunstige verhouding tussen verschillende eicosanoiden verantwoordelijk is voor het desastreuze beloop, namelijk een overmaat aan thromboxaan A2, een tekort aan prostaglandine E2 en een relatief tekort aan prostaglandine I2.

In hoofdstuk 8 wordt het effect van toediening van prostaglandine E2 op het sterftepercentage gemeten. Het sterftepercentage is 40% lager wanneer prostaglandine E2 wordt toegediend. Wanneer tevens de thromboxaan A2 synthese wordt geremd, gaat het sterftecijfer met 60% omlaag ($p < 0.05$).

In hoofdstuk 9 wordt het effect van toediening van iloprost (een stabiel analogon van prostaglandine I2) op het sterftepercentage gemeten. Toediening van iloprost vermindert het aantal sterfgevallen met 50%, terwijl een verdere reductie van het aantal sterfgevallen wordt bereikt door tegelijkertijd de synthese van thromboxaan A2 te remmen. Wanneer namelijk gelijktijdig flunarizine (een calcium entry blokker met een remmend effect op thromboxaan A2) wordt toegediend, overleeft 60% van de dieren, terwijl met dazmegrel (een sterke thromboxaan A2 synthese remmer) 90% van de ratten de eerste 48 uur overleven ($p < 0.01$).

In hoofdstuk 10 worden de effecten van een lang werkend somatostatine analogon (SMS 201-995) bestudeerd. Wanneer SMS 201-995 vroeg in het beloop van acute pancreatitis wordt toegediend, verbetert de overlevingstijd van de ratten significant. SMS 201-995, een hormoon zonder vervelende effecten, veroorzaakt een zesvoudige verhoging van circulerende plasmaspiegels van 6-keto-PGF1alpha, terwijl thromboxaan B2 spiegels met 177% toenemen. Het is aannemelijk dat somatostatine prostaglandine I2 als uiteindelijke mediator heeft, waarmee de belangrijke thromboxaan A2/prostaglandine I2 ratio ten gunste van prostaglandine I2 beinvloed wordt.

In hoofdstuk 11 worden alle resultaten besproken. Dit proefschrift geeft aan dat eicosanoiden het beloop van acute necrotizerende pancreatitis in ratten beinvloeden. De verhouding tussen thromboxaan A2 en prostaglandine I2 is met name belangrijk. Het lijkt met deze gegevens in de hand verantwoord om te kijken of in acute pancreatitis bij de mens eveneens een wanverhouding in eicosanoiden bestaat. Dat zou een interessant veld voor klinische research openen. De balans tussen thromboxaan A2 en prostacycline kan hersteld en verbeterd worden door remming van de thromboxaan A2 synthese in combinatie met een stimulatie van prostaglandine I2 door SMS 201-995 of toediening van iloprost.

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Rob Meijer (Laboratorium voor Experimentele Chirurgie) heeft de histologische coupes vervaardigd.

Pim van Schalkwijk (Laboratorium voor Experimentele Chirurgie) verrichtte de amylase en lipase bepalingen.

Medewerkers van het Laboratorium voor Interne Geneeskunde II verzorgden de Natriumtaurocholaat-oplossingen.

Pieter Uitterlinden (Laboratorium voor Interne Geneeskunde III) verrichtte de insuline bepalingen.

Drs C.J. Tinga (afdeling Pathologische Anatomie, Bronovo Ziekenhuis, 's Gravenhage) beoordeelde de vele histologische coupes, ook voor een patholoog-anatoom een inspannend werk.

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CURRICULUM VITAE

Bart van Ooijen werd op 11 mei 1956 te Rotterdam geboren. Van 1968-1974 volgde hij de middelbare schoolopleiding, aanvankelijk in Zwijndrecht (1968-1969) en Dordrecht (1969-1970), vervolgens in Verwoerdburg (Zuid-Afrika, 1970-1971) en, opnieuw, Dordrecht (1971-1974). In Dordrecht werd in 1974 aan het Christelijk Lyceum het einddiploma Gymnasium-β behaald. In datzelfde jaar werd de studie geneeskunde aangevangen aan de Erasmus Universiteit te Rotterdam. Voor het doctoraal examen slaagde hij in 1978, voor het artsexamen in december 1979. Vrijwel aansluitend werkte hij tot eind december 1981 als algemeen arts in het Gelukspan Community Hospital te Radithuso in Bophuthatswana. Sinds 1 januari 1982 is hij in opleiding tot algemeen chirurg in de Diakonesseninrichting "Bronovo" te 's Gravenhage (opleider: tot 1 januari 1983 dr A. Tammes, na 1 januari 1983 dr H.L. Kalsbeek).