FIBRINOLYTIC THERAPY IN GENERALIZED PERITONITIS TO PREVENT INTRAABDOMINAL ABSCESS FORMATION

Harm van Goor

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An experimental and clinical study

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Een wetenschappelijke proeve op het gebied van de Medische Wetenschappen

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is greatfully acknowledged.

To the memory of my father

CONTENTS

ABBREVIATIONS

ANOVA	analysis of variance
APACHE II	acute physiology and chronic health evaluation
APC	activated protein C
ATCC	American type culture collection
AT III	antithrombin III
AU	arbitrary units
BHI	brain heart infusion
cfu	colony forming units
CI	confidence interval
CT	computed tomography
ELISA	enzyme linked immuno sorbent assay
FbDP	fibrin degradation products
HMWK	high molecular weight kininogen
ICU	intensive care unit
IL	interleukin
IU	international units
IVGTT	intravenous glucose tolerance test
MHPC	methyl hydroxy propyl cellulose
MODS	multiple organ dysfunction syndrome
MOF	multiple organ failure
PAI	plasminogen activator inhibitor
PAP	plasmin-α_2-antiplasmin
RLS	Ringer's lactate solution
rtPA	recombinant tissue-type plasminogen activator
sctPA	single chain tissue-type plasminogen activator
SEM	standard error of the mean
TAT	thrombin-antithrombin III
tctPA	two chain tissue-type plasminogen activator
TF	tissue factor
TNF	tumor necrosis factor
TPFI	tissue factor pathway inhibitor
uPA	urokinase-type plasminogen activator

CHAPTER 1

GENERAL INTRODUCTION

▲INTRODUCTION

Infection of the abdominal cavity is a serious and potentially life-threatening condition. When untreated, about 90% of all patients with intra-abdominal infection die from sepsis[1]. The conventional approach to manage intra-abdominal infection is elimination of the source of peritoneal contamination, removal of purulent material, parenteral administration of antibiotics and haemodynamic and respiratory support. This approach results in complete recovery of most patients with intra-abdominal infection. However, this combined treatment may fail in patients with severe intra-abdominal infection, caused for example by perforation of the colon, leakage of intestinal anastomosis and pancreatic necrosis. Mortality rate of patients with these conditions is still high, varying from 20% to 60%[2-5]. Many patients have persistent or recurrent intra-abdominal infection and eventually died due to multiple organ dysfunction syndrome[5,6].
The disappointing outcome in patients with these severe forms of intra-abdominal infection has stimulated the search, in the past 20 years, for alternative treatment. At least three new operative methods have been proposed in an attempt to reduce high mortality in this group of patients[7]. Common purpose of these new operative methods was early aggressive removal of all intra-abdominal material, such as fibrin, tissue necrosis, and visceral content, that would potentiate intra-abdominal infection.

The first new operative method was radical peritoneal debridement e.g. elimination of all adherent fibrinous deposits on peritoneal surfaces. In the original nonrandomized trial of peritoneal debridement, mortality was extremely low[8]. A subsequent randomized study did not show benefit from extensive debridement of fibrinous deposits[9].

The second development was continuous postoperative peritoneal lavage[10-14]. In this technique, the problem inciting intra-abdominal infection is corrected, the abdomen is washed with normal saline solution, and drains are placed in the paracolic gutters and other accessible areas for infusion of peritoneal dialysis solution. The dialysis solution is evacuated from the abdomen through strategically positioned drains with the aid of gentle suction. The aim is to wash out infectious material, including bacteria, to prevent further intra-abdominal pus collections. One half of the studies reported in the literature indicates improved survival with this technique; the other half refutes such a benefit[15].

The third technique involved variations of the "open abdomen" approach for treatment of intra-abdominal infection[16-22]. In the "open" treatment of intra-abdominal infection, the abdominal cavity is treated as if it is an abscess cavity. After elimination of the source of infection, the abdominal incision is left open, providing free drainage of exudates and free access to the peritoneal cavity. Variations of this technique are the "semi-open" treatment and the method of "planned relaparotomy". In the "semi-open" treatment, the abdomen is temporarily closed without tension by artificial material, such as Marlex® mesh, Ethizip®, or Velcro® analogue, to prevent evisceration[23-26]. In the method of "planned relaparotomy", the abdominal cavity is re-explored at regular intervals (24 to 48 hours) to remove fibrin, necrotic tissue and other material that potentiate infection[26-32]. Several authors have demonstrated reduced mortality associated with the "open abdomen" approach or its variations. However, evaluation of these studies is difficult since they were nonrandomized, and heterogeneous patients groups were examined[7,21,22,32].

Although all these new operative methods were promising initially, they failed to gain wide acceptance, because improved outcome was not evident. Furthermore, these procedures were accompanied by serious intra-abdominal complications, such as haemorrhage, fistula formation and abdominal wall hernia[26,31,33-36].
Analogous to new operative methods, newer antibiotics, such as third generation cephalosporins or imipenem, monoclonal antibodies, such as human monoclonal antibody against endotoxin (HA-1A), and improvements in cardiovascular, respiratory, and renal support, have initially shown promising results, but have not evidently improved prognosis of patients with severe intra-abdominal infection in the past 10 to 20 years[7,37-41].
So, a new search for alternatives or adjuvants to the present treatment seems warranted.

▲THE ROLE OF FIBRIN AND FIBRINOLYSIS IN INTRAABDOMINAL INFECTION

It is generally accepted that residual or recurrent intra-abdominal infection promotes the development and progression of organ dysfunction[5,6,42-45]. Progressive organ dysfunction, induced by intra-abdominal infection, has a poor prognosis[5,6]. Delayed treatment of intra-abdominal infection often does not reverse organ dysfunction, whereas early intervention is more successful[6,46-52]. Prevention of residual or recurrent intra-

abdominal infection most likely improves prognosis.
Fibrin plays a crucial role in localizing infection in the abdominal cavity[53-55]. Thereby, fibrin protects the host from overwhelming bacteraemia[54]. However, fibrin also protects bacteria against host defense and antibiotics, and thereby favours residual or recurrent intra-abdominal infection[53].
Prevention of intra-abdominal fibrin formation may prevent residual or recurrent infection, which in turn may improve prognosis, particularly of patients initially operated on for severe intra-abdominal infection. Mechanical removal of fibrin during the initial operation has not evidently shown to increase survival in patients with intra-abdominal infection[9]. This may be due to intra-abdominal complications, such as bleeding and fistula formation, introduced by this technique. Furthermore, immediate recurrence of intra-abdominal fibrin deposition after operation may be expected. Repeated mechanical removal of fibrin deposits, as in the method of "planned relaparotomy", has decreased the rate of residual intra-abdominal abscesses[30,32]. However, this method introduces marked morbidity, which may adversely effect patient prognosis[32,56]. The complications of this surgical technique, applied in patients with severe intra-abdominal infection, due to colonic perforation or anastomotic leakage, or infected pancreatic necrosis, are outlined in more detail in Chapter 6 and 7.
An alternative to mechanical removal is enzymatical removal of fibrin. The enzymatic degradation of fibrin is carried out by the natural fibrinolytic system. During intra-abdominal infection the fibrinolytic system seems to be impaired[57,58]. Impaired fibrinolysis may be responsible for persistence of intra-abdominal fibrin and thereby development of residual intra-abdominal infection. Conceivably, enhancement of fibrinolysis in the abdominal cavity may prevent development of residual infection[59].
In this thesis, we focused on fibrin formation and fibrinolysis in the abdominal cavity during intra-abdominal infection and the effect of enhancement of intra-abdominal fibrinolysis on residual intra-abdominal infection. The following objectives of study were formulated.

▲OBJECTIVES

1. What is the effect of intra-abdominal infection on fibrin formation and fibrinolysis in the abdominal fluid and plasma and how does the fibrinolytic

activity relate to peritoneal injury (Chapter 8 and 9)?

2 Does human recombinant tissue-type plasminogen activator (rtPA) -a fibrinolytic agent- effect the rat fibrinolytic system (Chapter 10)? What are the pharmacokinetics of human rtPA administered into the abdominal cavity of rats with intra-abdominal infection (Chapter 11)?

3 What are the effects of human rtPA on mortality and intra-abdominal abscess formation in rats with intra-abdominal infection (Chapter 12)?

4 Does combined treatment of human rtPA and gentamicin improve outcome in rats with intra-abdominal infection (Chapter 13)?

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CHAPTER 2

ANATOMY AND (PATHO)PHYSIOLOGY OF THE ABDOMINAL CAVITY

▲ANATOMY AND PHYSIOLOGY OF THE ABDOMINAL CAVITY

The abdominal cavity is the largest preformed extravascular space in the body. Normally the viscera within the abdominal cavity are in contact along their serosal surfaces. Therefore, the abdominal cavity contains less than 50 ml of clear yellow fluid. This fluid is normally sterile and has a specific gravity of less than 1.016 and a protein level of less than 30 g/l, most of which is albumin. The peritoneal fluid contains fewer than 3,000 cells/mm^3, with 50 percent macrophages, 40 percent lymphocytes, a few eosinophils and mast cells, and an occasional mesothelial cell[1].

The peritoneum lines the abdominal cavity as the parietal peritoneum and reflects onto the abdominal viscera as the visceral peritoneum. In adults, the area of peritoneal surface approximates that of the cutaneous surface, which is about 1.7 m^2. The peritoneum consists of a single layer of flat mesothelial cells, that resides on a basement membrane, which, in turn, overlies a bed of connective tissue consisting of fat cells, macrophages, fibroblasts, lymphocytes, a few mast cells, and collagen and elastic fibres[2]. The peritoneum is a smooth, translucent membrane. Most of this membrane behaves as a passive, semipermeable barrier to the bidirectional diffusion of water and low molecular weight solutes. Studies of dialysis patients have shown that the functional exchange surface of the abdominal cavity is approximately one square meter[3]. Factors that alter the fluid exchange are changes in the membrane area, in the splanchnic blood flow, and in the peritoneal membrane permeability[4,5].

Although the entire peritoneal surface participates in fluid and low molecular weight solute exchange, particulate material is cleared through stomata between specialised peritoneal mesothelial cells that overlie lymphatic channels on the diaphragmatic surface of the abdominal cavity[2,6-8]. These intercellular stomata correspond with fenestrations in the basement membrane, and together they serve as channels leading from the abdominal cavity to the underlying specialised diaphragmatic lymphatic vessels called lacunae (Figure 1)[7,9].

Fluid flows rapidly through the stomata into the lacunae with relaxation of the diaphragm, e.g. during expiration. Contraction of the diaphragm, during inspiration, empties the lymphatics into efferent ducts, aided by the simultaneous drop in intrathoracic pressure. One-way valves in the thoracic lymphatics prevent reversed flow. Injection of contrast material into the abdominal cavity has demonstrated a cephalad flow into the upper abdomen (suprahepatic and infrahepatic spaces), as well as into the pelvis and paracolic

gutters[10]. Movements into the upper abdomen probably represent flow into a region of low pressure produced by absorption of fluid and material by the diaphragmatic lymphatics and a suction effect caused by the pull of gravity on the upper abdominal viscera, particularly the liver, away from the diaphragm (Figure 2)[11]. Flow into the pelvis and the paracolic gutters is likely the effect of gravity in the prone or semi-upright position.

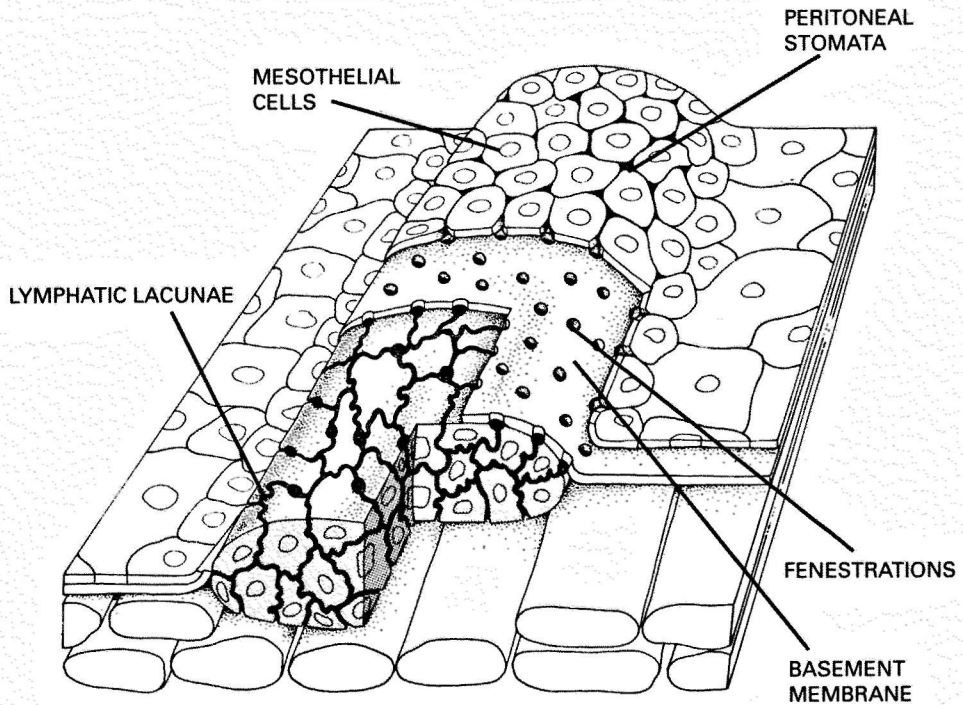

Figure 1. Schematic view of peritoneal stomata and underlying lymphatic tissue. Over the lymphatics, mesothelial cells are small, with stomata between the cells. Through these stomata and corresponding fenestrations of the basement membrane, the abdominal cavity communicates with lymphatic lacunae, which are lined by lymphatic endothelium.
(Modified from Allen L[2])

The size of the stomata (8 to 12 μm) determines the maximum size of particles that are readily absorbed from the abdominal cavity. Bacteria, which average 0.5 to 2 μm, pass easily through these stomata. After inoculation in the abdominal cavity, bacteria

are recoverable from the right thoracic lymph duct of dogs within six minutes and from the blood within 12 minutes[12]. Bacteria are not absorbed to any extent from nondiaphragmatic peritoneal surfaces.

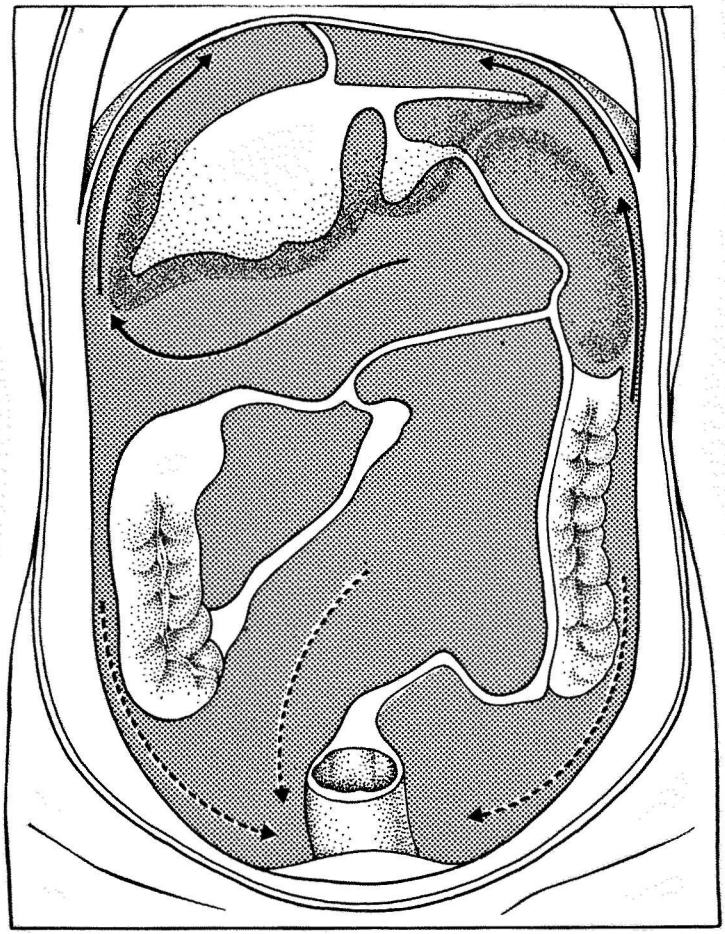

Figure 2. Circulation of fluid and particles in the abdominal cavity. Solid arrows indicate the direction of flow, generated by diaphragmatic movement and absorption of material from the diaphragmatic lymphatics. Dashed arrows demonstrate the effect of gravity in the upright position.
(Modified from Autio V[10])

Various factors can affect peritoneal clearance of particulate matter, such as bacteria (Table 1)[12-17]. Already in 1900, Fowler used the semi-upright position in nine patients with intra-abdominal infection to prevent the rapid absorption of "toxins" from the abdominal cavity[13]. In 1944, Steinberg showed that absorption of bacteria from the abdominal cavity was delayed by the upright position and accelerated by the head-down position[12]. In dogs, laparotomy with manipulation of the viscera, leading to ileus, causes a delayed clearance time of peritoneal graphite particles[14]. Phrenic neurectomy, with resultant diaphragmatic paralysis, initially delays absorption on the denervated site; the absorption is later accelerated as the muscles atrophy[14]. Increased intraperitoneal pressure accelerates the clearance of material from the abdominal cavity[15]. Depression of spontaneous respiration and increased intrathoracic pressure decrease efferent lymph flow and bacterial clearance from the abdominal cavity[16,17]. All these mechanical factors may greatly influence the ability of the abdominal cavity to handle bacterial inocula.

Table 1. Factors impairing mechanical clearance of fluid and particles from the abdominal cavity.

Factor	**Effect**
Posture (head up/pelvis down)	Impaired gravitational drainage
Hypoventilation (general anaesthetics, phrenic neurectomy)	Altered diaphragmatic pumping action
Mechanical ventilation, especially with positive end-expiratory pressure	Constant positive intrathoracic pressure
Ileus	Impaired intra-abdominal circulation
Ascites	Overwhelmed lymphatics

▲HOST RESPONSE TO INTRAABDOMINAL INFECTION

Local response

After bacterial contamination of the abdominal cavity, a complex series of events is initiated by the host in order to eradicate invading bacteria. These local host defense mechanisms are: (1) mechanical clearance of fluid and bacteria via the diaphragmatic lymphatics, (2) phagocytosis and destruction of bacteria by phagocytic cells, and (3) sequestration of bacteria.

(1) After intra-abdominal inoculation, bacteria begin to disappear from the abdominal cavity immediately, even before the influx of phagocytic cells[18]. As mentioned before, bacteria are recoverable from the right thoracic lymph duct within six minutes and from the blood within 12 minutes after intra-abdominal inoculation[12]. Bacteria are transported by the abdominal fluid circulation via the paracolic gutters to the left and right subdiaphragmatic spaces (Figure 2)[10,19]. These observations suggest that the first line of defense in the abdominal cavity is physical removal, whereby bacteria are carried cephalad by the abdominal fluid circulation, absorbed into the diaphragmatic lymphatics, and then carried to the bloodstream.

(2) The second line of defense is phagocytosis and killing of bacteria by phagocytic cells. Influx of phagocytic cells into the abdominal cavity is part of the initial peritoneal response to bacterial contamination, which is further characterized by vasodilatation and fluid exudation[20]. Peritoneal macrophages predominate in the abdominal cavity in the first four hours of infection. Thereafter, neutrophils are the most important phagocytic cells for 48 to 72 hours[18]. After about 48 to 72 hours macrophages again predominate in the inflammatory process. Influx of phagocytes into the abdominal cavity is mediated by bacterial cell products (e.g. lipopolysaccharide), complement cleavage products (specifically C5a), inflammatory proteins (kinins, histamine) and cytokines such as tumor necrosis factor (TNF) and interleukins (IL-1, IL-6, IL-8)[20-31]. Many of these cytokines are released by macrophages. Lipopolysaccharide, derived from gram-negative enteric bacteria, is a particularly strong stimulus for release of cytokines by peritoneal macrophages. These enteric bacteria are most commonly found in intra-abdominal infectious processes.

After chemotaxis, invading organisms must be ingested and destroyed Particularly, encapsulated micro-organisms, are able to resist ingestion by human phagocytes and must be opsonized with a sufficient amount of specific antibody to be ingested[32] Activated C3 and IgG are the best-characterized and most important opsonins On contact of the opsonized bacteria with the phagocyte, fusion processes are initiated, followed by degranulation of antimicrobial products, that eventually kill the bacteria[33 36]

(3) The third line of defense is sequestration of bacteria Fibrin plays a crucial role in walling off infection by incorporating bacteria in its matrix and by creating a physical barrier against dissemination through formation of intra-abdominal adhesions[19 23 37 38] The entrapment of bacteria during the clotting process is highly effective Fibrin also impairs the clearance of bacteria from the abdominal cavity by occluding the diaphragmal stomata[39] Furthermore, local fibrin deposition seems to stimulate the inflammatory response by increasing vascular permeability and chemotaxis of neutrophils and monocytes[40-42] By all these actions, fibrin protects the host against early systemic spread of bacteria and subsequent death[37]

If these peritoneal defense mechanisms fail in controlling intra-abdominal infection, the peritoneal cavity is flooded with infectious material Under these circumstances, the peritoneal defense mechanisms may have detrimental effects on the host Five interacting mechanisms are responsible for the failure to control infection (1) fluid exudation into the abdominal cavity, (2) impairment of the immunological defense mechanisms, (3) adjuvant substances, (4) bacterial synergism, and (5) fibrin

(1) The influx of large amounts of protein-rich exudate into the abdominal cavity produces massive third-space fluid shifts These exudates impair bacterial opsonization by diluting opsonins and reduce the ability of phagocytic cells to reach and to phagocytose the bacteria[43] Absorption of these intraperitoneal exudates and bacteria is disturbed by occlusion of the diaphragmal stomata

(2) The immunological defense mechanisms are impaired by dilution of chemotaxic factors and opsonins, by adjuvant substances, and by fibrin Adjuvant substances, such as bile salts, gastric mucin, faeces, barium sulphate, necrotic tissue, blood components, and foreign bodies, inhibit the migration and killing capacity of neutrophils

and result in premature release of oxygen radicals by these cells[18,44-54]. It has been demonstrated that fibrin inhibits phagocytic killing of *Escherichia coli (E. coli)* by neutrophils and impedes macrophage migration[55,56]. This inhibition is related to impairment of phagocytosis rather than irreversible damage to the killing system of the neutrophil[55]. Impairment of phagocytosis might be due (i) to the physical entrapment of neutrophils preventing locomotion, (ii) to reduced opsonization of bacteria in a fibrinous environment, and (iii) to premature release of lysosomal enzymes in the presence of activated complement components along fibrin strands.

(3) Adjuvant substances not only impair immunological defense mechanisms, but promote bacterial growth. Haemoglobin, for example, enhances bacterial growth and increases bacterial virulence[6]. In vitro addition of iron to a culture medium enhances growth of *E. coli*[49].

(4) More so than a monomicrobial infection, a polymicrobial intra-abdominal infection enhances lethality and the formation of residual abscesses. Herein, synergism between anaerobic and aerobic bacteria plays an important role. Especially the synergism between *Bacteroides fragilis* and *E. coli* has been studied in vitro and in vivo[57-62]. The possible mechanisms of their synergy are: (i) the ability of one species to provide growth nutrients for its bacterial partner, (ii) the ability of one species to impair host defenses, permitting its co-pathogen to survive and exert its intrinsic virulence, and (iii) the ability of the species to optimize local environment, thereby enhancing bacterial proliferation.

(5) Fibrin entraps bacteria and provides a protected environment, in which bacteria may proliferate almost unaffected by neutrophils. As mentioned, fibrin also impairs neutrophil function[55]. Fibrin blocks diaphragmal stomata and thereby prevents absorption of intra-abdominal fluid and bacteria[39].

Impairment of function of neutrophils due to bacteria, fibrin, and adjuvant substances, is accompanied by premature extracellular release of neutrophil enzymes[63]. During intra-abdominal infection, these enzymes are present in such concentrations in the peritoneal fluid, that the neutralizing activity of inhibitors of these enzymes, such as alpha-1 protease inhibitor (complexing leukocyte elastase), is insufficient[64]. Neutrophil enzymes are capable of damaging viable tissue, resulting in necrosis. In addition to

the enzymes liberated by neutrophils, bacteria themselves produce a number of exoenzymes, such as hyaluronidase and lipase, that cause tissue destruction[65]. In such an environment, characterized by hypoxia and a low pH, neutrophil function is further impaired and bacterial growth is stimulated[66-74]. Tissue necrosis, fibrin and bacteria augment the severity of the inflammatory process with continuous influx of neutrophils and macrophages. This process may readily become self-perpetuating, even in the absence of bacteria.

Systemic response

The systemic response to intra-abdominal infection mimics the body's response to trauma in general and includes the rapid release of catecholamines, increased secretion of adrenocortical hormones and secretion of aldosterone and antidiuretic hormone[11]. Of particular importance are the haemodynamic and cytokine responses to intra-abdominal infection.

The haemodynamic alterations observed in patients with intra-abdominal infection are mainly due to hypovolaemia. Diffuse intra-abdominal infection has been likened to a total burn surface of 50 percent in terms of its effect on fluid shifts. A massive fluid shift into the peritoneal tissues and cavity produces hypovolaemic shock with a reduced cardiac index, elevated peripheral vascular resistance, and increased peripheral oxygen extraction. After fluid resuscitation, patients with severe intra-abdominal infection demonstrate the more classic "septic" (hyperdynamic) response: elevated cardiac output, reduced peripheral vascular resistance, and a narrowing arterio-venous O_2 content difference[75,76].

As proposed for the local response to infection in the abdominal cavity, the stimulated release of products of cells of the monocyte-macrophage lineage also appears to be responsible for the characteristic systemic host response observed in patients with intra-abdominal infection. Intravenous infusion of TNF into experimental animals mimics the haemodynamic alterations and lactate acidosis that follow the administration of bacterial endotoxin[77]. Both TNF and IL-1 cause fever and neutrophilia, and IL-6 initiates the acute-phase protein response characteristic of infection[77-79]. The central role of these cytokines in mediating the systemic host response is further illustrated by the ability of antibodies directed against either TNF or IL-6 to abrogate the adverse effects of endotoxaemia[80,81].

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CHAPTER 3

INTRAABDOMINAL INFECTIONS: DEFINITIONS AND AETIOLOGY

▲INTRAABDOMINAL INFECTION

Intra-abdominal infection or intra-abdominal sepsis is defined as an inflammatory process of bacterial origin in the abdominal cavity.
There are two major manifestations of intra-abdominal infection: bacterial peritonitis or infection of the peritoneum, and intra-abdominal abscess, in which infection has become walled off from the remainder of the abdominal cavity[1].

▲PERITONITIS

Peritonitis is defined as an inflammation of (part of) the peritoneum. Peritonitis may be divided in bacterial, chemical, ischaemic or mechanical peritonitis, based on the type of peritoneal injury. Another classification is that of a local or a generalized (diffuse) peritonitis, based on the extent of peritoneal inflammation. Other terms as severe, suppurative or fulminant peritonitis often refer to serious forms of generalized peritonitis, in which the abdomen is flooded with pus, and patients are critically ill.
The classification into (1) primary peritonitis, (2) secondary peritonitis, and (3) tertiary peritonitis, based on aetiology, is most commonly used[1-3].

(1) Primary peritonitis is defined as a diffuse bacterial infection of the abdominal cavity in which there is no obvious source, such as a perforated viscus.
Primary peritonitis classically occurs in young girls and is caused by *Streptococcus pneumoniae*[4,5]. The incidence of primary peritonitis in children has decreased after antibiotics became available[5,6]. This form of peritonitis is being recognised with increasing frequency in the adult population. In adults, the presence of alcoholic cirrhosis and ascites is the most frequent underlying risk factor[7,8]. Cirrhotic ascites predisposes to infection because of reduced total protein and complement levels, which result in impaired bacterial opsonization[9]. In patients with cirrhosis, monomicrobial infections with enteric bacteria, particularly *Escherichia coli* (*E. coli*), predominate, whereas a polymicrobial flora is present in less than 10 percent of the cases[10].
Rare forms of primary peritonitis are tuberculous peritonitis (caused by *Mycobacterium* species), abdominal actinomycosis and amoebic peritonitis.

(2) Secondary peritonitis is defined as a peritoneal inflammation due to perforation of a hollow viscus or transmural necrosis of the gastrointestinal tract. Common causes of secondary peritonitis are perforated appendix, perforated duodenal ulcer, perforated sigmoid colon due to diverticulitis, volvulus or cancer, strangulation obstruction of the small intestine, and postoperative peritonitis due to anastomotic leakage. Pancreatic necrosis is also considered as a cause of secondary peritonitis.
The spectrum of microorganisms inoculating the peritoneum after perforation of the gastrointestinal tract depends on the level of perforation as well as the pathologic process leading to the perforation. Stone et al. did not find bacteria in the abdominal cavity when a normal human duodenum was perforated[11]. Gram-positive bacteria are predominantly released in perforations of the upper gastrointestinal tract. However, patients who have been taken antacids or H_2-receptor blockers have greater numbers of gram-negative bacteria in their stomach before perforation [12,13]. Bacterial peritonitis due to a perforation of the distal small intestine or the colon is almost always polymicrobial, containing a mixture of aerobic and anaerobic bacteria[14]. Most common aerobic and anaerobic species, that are found in the abdominal cavity of patients with secondary bacterial peritonitis, are *E. coli* and *Bacteroides fragilis (B. fragilis)*, respectively[14,15].

A special form of secondary bacterial peritonitis, that often is categorized as primary peritonitis, is peritonitis in patients on continuous ambulatory peritoneal dialysis. The infection is commonly from exogenous sources, usually by *Staphylococcus epidermidis (St. epidermidis)*[1,16]. Treatment differs from other forms of secondary peritonitis as to the elimination of the source of infection, i.e. the dialysis catheter. Primarily, antibiotics are administered, while the catheter remains in situ.

(3) Tertiary peritonitis is defined as a persistent inflammation of the abdominal cavity, associated with multiple organ dysfunction and general depression of the immune system. Patients with tertiary peritonitis are unable to localize infection, whether due to impaired host defense mechanisms or overwhelming infection[11].
St. epidermidis, *Pseudomonas* species and *Candida* species are the predominant microorganisms recovered from these patients[17,18]. The common microorganisms isolated from patients with secondary bacterial peritonitis, *E. coli* and *B. fragilis*, are only occasionally found in these patients. In a number of patients with tertiary peritonitis,

no bacteria are isolated from the abdominal cavity. The bacteria comprising the flora of tertiary peritonitis have classically been considered innocuous colonizers rather than true pathogens. However, the fact that these bacteria are true invasive pathogens rather than merely colonising bacteria is clearly demonstrated by their ability to become blood-borne. It is therefore likely that a relatively immunocompromised host contributes to their pathogenicity.

Several lines of evidence suggest that the bacteria found in the abdominal cavity of patients with tertiary peritonitis originate from the gastrointestinal tract[19,20]. In experimental models, the migration of enteric bacteria through the intestinal wall, a process called bacterial translocation, into sterile inflammatory processes in the abdominal cavity has been shown[19,21]. A study in patients with multiple organ dysfunction has demonstrated a correlation between upper gastrointestinal tract flora and invasive infection with the same organisms[17]. Moreover, it has been reported that small intestinal bacterial overgrowth, which is an important cause of bacterial translocation, depresses the immune system[22,23]. This event may then initiate a vicious circle leading to further bacterial translocation and a worsening of the immune-compromised state of the host.

▲INTRAABDOMINAL ABSCESS

Intra-abdominal abscesses are well-defined collections of pus which are walled off from the rest of the peritoneal cavity by inflammatory adhesions, loops of intestine and their mesentery, the greater omentum, or other abdominal viscera. Intra-abdominal abscesses represent the successful result of normal host defense mechanisms in the abdominal cavity. These abscesses arise in two situations: (i) after resolution of diffuse intra-abdominal infection in which a loculated area of infection persists and evolves into an abscess, and (ii) after perforation of a viscus or an anastomotic breakdown that is successfully walled off by peritoneal defense mechanisms.

Intra-abdominal abscesses almost always consist of both aerobic and anaerobic bacteria[15,24]. As in secondary bacterial peritonitis, most common bacteria isolated from the abscess cavity are *E. coli* and *B. fragilis*[15]. The abscess cavity is a microenvironment, characterized by hypoxia, low pH, large numbers of bacteria in the stationary phase, and high concentrations of bacterial toxins and proteases[12,25]. In such an environment host defense mechanisms are impaired and antibiotic efficacy is reduced[26-28].

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CHAPTER 4

SURGICAL TREATMENT OF INTRAABDOMINAL INFECTION

▲INTRODUCTION

Surgery is the primary treatment modality to manage intra-abdominal infection, caused by perforation of a hollow viscus or transmural necrosis of the gastrointestinal tract, or caused by pancreatic necrosis. In 1926, the operative management of intra-abdominal infection has been defined by Kirschner as 'Ausshaltung der Infektionsquelle, Beseitigung des Exsudates, Behandlung der Bauchhölle mit Desinfektionsmitteln und Ableitung des Exsudates'[1]. Nowadays the elimination or control of the infectious source, and the reduction of peritoneal contamination by debridement and lavage are still the cornerstones of primary operative treatment[2].

▲SOURCE OF INFECTION

In general the source of infection is eliminated, avoiding continuous peritoneal soiling, by resection or closing the perforated viscus, or resection of necrosis.
For pathology located in the large intestine, resection is usually performed, followed by creation of a proximal enterostomy (Hartmann's procedure). In this location, primary anastomosis is avoided because of the high risk of anastomotic dehiscence[3].
The risk associated with primary anastomosis of the small intestine, following resection of a diseased segment, is considered much lower. However, if peritoneal soiling is particularly extensive, resection plus proximal and distal enterostomy is advocated[4,5].
Suturing a perforation is mainly performed today for perforated peptic ulcer. Additionally, it may be considered for single, small, foreign body or traumatic perforations of the small intestine, diagnosed early[5].

▲DEBRIDEMENT AND LAVAGE

Reduction of peritoneal contamination by debridement and intraoperative lavage is intended to prevent residual infection. As discussed in Chapter 2, adjuvant substances as foreign material, necrotic tissue, fibrin, bile, blood or intestinal content enhance the severity of the infectious process by stimulation of bacterial growth and impairment of neutrophil and macrophage function.

"Radical debridement" -eliminating all adherent fibrinous deposits on peritoneal surfaces- was advocated by Hudspeth[6]. In his series of 92 patients with peritonitis treated by "radical debridement", only one intra-abdominal abscess developed, and all patients recovered. However, in a prospective randomized trial of 46 patients with diffuse peritonitis, receiving either standard surgical therapy or "radical debridement", Polk and Fry did not find better results from the radical operation[7]. On the contrary, excessive bleeding from serosal surfaces and intestinal perforations was more often encountered attributed to mechanical removal of fibrin.
Intraoperative lavage has become a standard procedure[2,8]. However, in clinical studies the efficacy of intraoperative lavage has not well been documented[8,9]. Intraoperative lavage is applied to reduce the quantity of bacteria and to remove adjuvant substances, thus supporting the local host defense mechanisms[10,11]. The fear of disseminating bacteria by lavage is probably unfounded, since it has been demonstrated in animal experiments that, even without irrigation, locally applied particles spread rapidly throughout the peritoneal cavity by the abdominal fluid circulation[12].
The question whether the lavage solution should contain antibiotic or antiseptic preparations is still widely discussed. From recent reviews on this subject, it appears that intraoperative lavage with antibiotics or antiseptics does not improve outcome in patients with intra-abdominal infection, who already receive appropriate systemic antibiotics[8,9].

There is a great variance in surgical strategies to be followed after elimination of the source, debridement and intraoperative lavage, especially in patients with severe intra-abdominal infection. These strategies vary from a conservative "wait and see" policy to the most aggressive method of "planned relaparotomy". Four strategies may be distinguished: (1) "wait and see" policy with or without tube drainage, (2) continuous postoperative peritoneal lavage, (3) "open" drainage (laparostomy), and (4) "planned relaparotomy"[2].

(1) In the "wait and see" policy surgeons rely on host defenses, eradicating bacteria and adjuvant substances, that remain intraperitoneally after the first operation. However, in severe forms of intra-abdominal infection, host defenses are not likely to eradicate remaining bacteria and adjuvant substances. This is illustrated by the high incidence of residual intra-abdominal infection in patients with diffuse peritonitis, treated according

to the "wait and see" policy[13-16].
In the "wait and see" policy, surgeons also rely on clinical signs of residual or recurrent intra-abdominal infection for the decision whether or not to re-explore the abdominal cavity (i.e. "on demand" relaparotomy).
Clinical signs of residual intra-abdominal infection are often blunted in patients, who are critically ill and cared for in intensive care units. Furthermore, diagnostic investigations, such as ultrasound or computed tomography, for the detection of residual infection are often not conclusive or impossible to perform. Therefore, most "on demand" relaparotomies are performed on the basis of, otherwise unexplained, progressive organ dysfunction or bacteraemia[17-22]. These relaparotomies are technically difficult to perform, have a high morbidity, and often do not reverse organ dysfunction, even when infectious foci are encountered and drained[18,19,23,24].
Tubes are meant to drain residual blood, pus, peritoneal fluid, and necrotic material in the postoperative period[25]. In diffuse intra-abdominal infection the role of tube drainage, if any, seems to be a minor one[26,27]. This is predominantly caused by the rapidity in which drains will be sealed off by fibrin deposition or in which fibrinous drain tracts are formed, that do not communicate with the abdominal cavity as such[28-31]. Beside the fact that drains simply do not work from the beginning, tubes placed intraperitoneally may cause visceral and vessel wall erosion with fistula formation and bleeding[2,32]. Moreover, drains like other foreign bodies impair neutrophil function and potentiate infection[33]. The risk of being an access route for external bacteria into the abdominal cavity, although present, should not be overestimated in this situation[34,35]. The only indications for intra-abdominal drains are evacuating well-defined abscesses, and offering a preferential pathway for the escape of visceral secretions, such as bowel content, pancreatic juice and bile[36].

(2) The concept of continuous postoperative peritoneal lavage is not a new one, but has recently received more attention as a means to reduce residual intra-abdominal infection[37-46]. At the time of initial surgery, several peritoneal tubes are placed in "strategic" positions to irrigate the abdominal cavity in the postoperative phase[37,38]. Lavage with 10 to 20 litres of dialysis solution is advised to ensure dispersion of the solution throughout the abdominal cavity and to prevent fluid loculi. Antibiotics added to the lavage fluid might increase the therapeutic effect[37,40,41]. Silenas et al., and Beger and associates, emphasized the mechanical action of continuous postoperative peritoneal

lavage, detaching necrosis, debris, and fibrin from surrounding tissues[39,47].
Despite the potentially beneficial effects of continuous postoperative peritoneal lavage, reports on this method show conflicting results[42-45]. Reduction in postoperative abscess formation is reported in only half of the, mainly nonrandomized, comparative studies[43]. The few prospective randomized studies have small numbers of patients in the treatment groups, which do not allow for reaching conclusions[43,44,46]. Only in the treatment of necrotizing pancreatitis, continuous postoperative peritoneal lavage was found to be a valuable adjunct to debridement of necrotic pancreas[47].
In several reports, the disadvantages of continuous postoperative peritoneal lavage are emphasized: drains seal off in short time, bowel perforation, and fluid and electrolyte disturbances[43-45].

(3) The third strategy, leaving the abdomen open (laparostomy), was initially applied in France[48,49]. The principle of this technique is to treat the whole abdominal cavity as if it is an abscess cavity. The laparostomy should provide for free drainage of exudates and simple access to the abdominal cavity after the first operation[50-54]. In practice, free drainage becomes impossible within 1-2 days as a consequence of rapid adhesion formation by fibrin deposition. For the same reason safe access to all parts of the abdomen will be difficult[31,55].
The main advantage of this technique seems to be decompression of the abdomen. The relief of the elevated intra-abdominal pressure improves ventilation, splanchnic circulation, bacterial clearance, cardiac output and renal function[55-61]. Particularly during the first postoperative days, abdominal decompression might benefit the patient.
Leaving the abdomen open causes the risk of evisceration of the abdominal content, fistula formation and abdominal wall hernia, which in conjunction with the nursing problems, are considered major limitations of this technique[57,62-66]. These drawbacks have generated the application of alternative methods combining abdominal decompression with covering of intra-abdominal organs by, for example, Marlex® mesh or skin closure[58,67,68]. Although these methods diminish complications and provide abdominal decompression, drainage of peritoneal fluid is still inadequate due to rapid fibrin deposition and adhesion formation.

(4) The concept of "planned relaparotomy" has evolved from the technique to leave the abdomen open[55,69-72]. Re-exploration of the abdominal cavity is performed on a

24-48 hour basis to remove residual infectious material, such as necrosis, fibrin, and bacteria and to prevent fluid loculi. The abdomen may be left open or temporarily closed by means of a Zipper® or Velcro® analogue, to relieve intra-abdominal pressure and to facilitate re-explorations[73-75]. In contrast to the "on demand" relaparotomy, which is often complicated because of the firm adhesions encountered, planned relaparotomies deal with loose and easy to separate fibrinous attachments, especially in the beginning[18,55,71,76,77].

The success of this approach has been attributed to improved elimination of the bacterial inoculum, necrotic material and fibrin, and the possibility to early detect and correct intra-abdominal complications[75]. Favourable results in terms of mortality and residual abscess formation of this aggressive therapy compared to more conventional surgical therapies have been reported. However, prospective randomized studies are still lacking[62,66-71,74,78]. More recently the disadvantages of this treatment were brought under attention: unnecessary re-explorations, bleeding due to frequent manipulation of the abdominal content and removing fibrin from serosal surfaces, "spontaneous" fistula formation, interference with anastomotic healing by detaching adhesions and removing fibrin deposits, excessive fluid and protein losses, and large abdominal wall defects[42,62,79,80]. In two studies of 24 patients with the most severe forms of generalized peritonitis (massive faecal peritonitis due to perforation of the colon and postoperative anastomotic leakage) and 10 patients with infected pancreatic necrosis, respectively, treated by "planned relaparotomy" with meticulous removal of fibrin and necrosis, the complications of this treatment modality were analyzed (Chapter 6 and 7)[79,80]. Only one patient developed a residual intra-abdominal abscess, but the number of other complications, such as intra-abdominal bleeding, bowel perforation and enteric fistula, was high. Intra-abdominal infection was successfully treated after a median number of three planned relaparotomies, as assessed by bacterial cultures of the peritoneal fluid. The number of intra-abdominal complications was related to the number of planned relaparotomies and to patient outcome. Some of these findings are in concordance with those of Schein et al.[62]. In their series of 52 patients with severe intra-abdominal infection, none of the patients had residual or recurrent infection, six (12%) had massive haemorrhage, 16 (31%) developed gastrointestinal fistulas, and in 12 (23%) an abdominal wall defect persisted. In a series of 117 patients, reported by Wittmann and associates, 10% of the patients had severe haemorrhage, needing laparotomy, and the incidence of suture line disruption and bowel perforation was 19%[74].

It may be concluded that the ideal operative approach for patients with severe forms of intra-abdominal infection has not been established yet More conventional approaches, such as the "wait and see" policy or continuous postoperative peritoneal lavage, do not seem to prevent residual or recurrent intra-abdominal infection and are associated with a high mortality The method of "planned relaparotomy" seems to decrease the rate of residual intra-abdominal infection However, this method has a high complication rate and improvement in patient survival has thusfar not evidently been demonstrated

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CHAPTER 5

INTRAADBOMINAL FIBRIN FORMATION AND FIBRINOLYSIS

▲INTRODUCTION

Every insult to the peritoneal cavity and especially the peritoneum will induce exudation of fibrinogen-rich fluid into the peritoneal cavity, which in turn results in intra-abdominal fibrin deposition[1,2]. The formation of fibrin is crucial in processes such as haemostasis, inflammation and tissue repair. However, fibrin has only a temporary role and must be resolved in order to resume normal tissue function. Degradation of fibrin is regulated by the fibrinolytic system. This system can be regarded as the physiological counterpart of the coagulation system[3].
In this chapter, the coagulation system, the fibrinolytic system, intra-abdominal coagulation and fibrinolysis in peritonitis, and the possibilities to modulate intra-abdominal fibrin deposition are outlined.

▲THE COAGULATION SYSTEM

The coagulation system comprises two pathways of action, i.e. the extrinsic or tissue factor (TF) dependent pathway and the intrinsic pathway. Activation of each of both pathways results in the generation of thrombin via the common pathway, which converts fibrinogen into fibrin (Figure 1). Fibrin monomers are formed by cleavage of small peptides (fibrinopeptides A and B) from the A-alpha and B-beta chains of fibrinogen by thrombin. Factor XIII, activated by thrombin, catalyses a process of cross-linking with formation of intermolecular isopeptide bonds of adjacent fibrin monomers. In this way, a matrix of fibrin is formed which will be stabilized by the ingrowth of cellular elements.
The extrinsic pathway is activated by complex formation between factor VII and TF, a protein, that is expressed at the surface of endothelial cells, mesothelium and macrophages upon stimuli, such as endotoxin[4-7]. Upon binding to TF, factor VII becomes highly susceptible to proteolytic activation by trace amounts of factor IXa and Xa, resulting in the formation of factor VIIa-TF complex[5,7]. The factor VIIa-TF complex activates factor X, which forms a complex with factor Va, calcium ions and phospholipid, which in turn converts prothrombin into thrombin[8,9].
Activation of the intrinsic pathway proceeds in vitro by interactions between (pre)kallikrein,

high molecular weight kininogen (HMWK) and factor XII, respectively[10,11]. Through activation of factor XII and XI, factor IX is activated, which forms a complex with its cofactor VIIIa, calcium ions and phospholipids. This complex cleaves factor X into Xa, which subsequently induces thrombin formation[12].

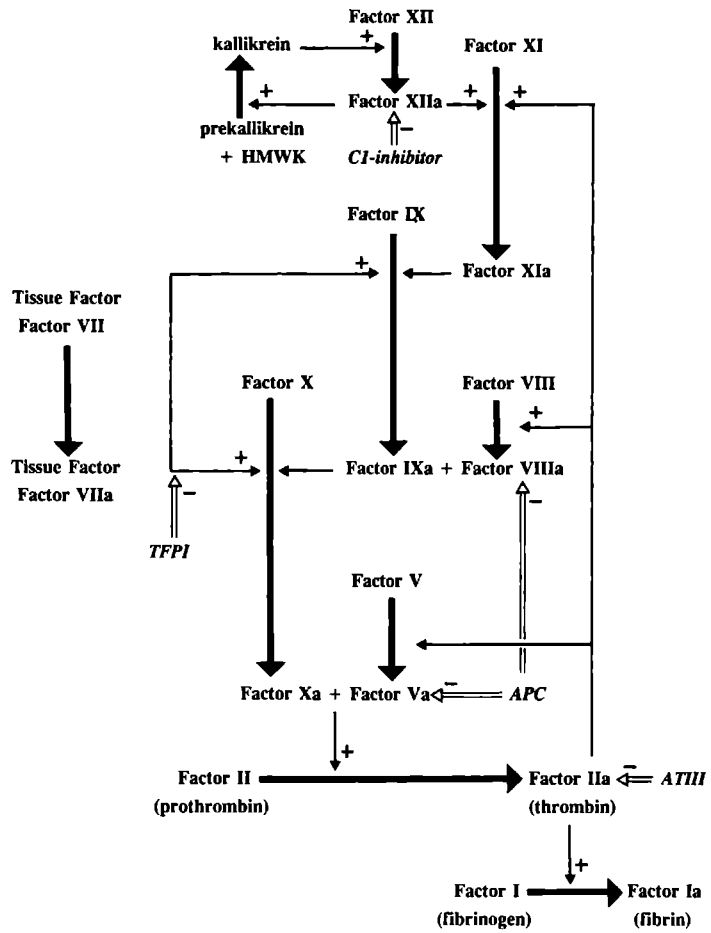

Figure 1. The coagulation cascade.

In plasma, the TF-driven pathway exclusively contributes to the basal level of coagulation activation in vivo[13]. Recent studies in healthy volunteers and non-human primates

revealed that, by the administration of endotoxin or tumor necrosis factor (TNF), the activation of the coagulation system is completely TF pathway dependent[14-16]. The intrinsic pathway might only play a role in conditions requiring an accelerated level of coagulation activity, such as trauma[17].
TF-mediated activation of coagulation is regulated by tissue factor pathway inhibitor (TPFI), which binds to the factor VIIa-TF-Xa complex, resulting in an inactive complex[18,19]. Activation of the intrinsic pathway is controlled by the protein C system, which is activated by thrombomodulin bound thrombin[20,21]. Activated protein C (APC) inhibits the process of coagulation by degradation of activated factors V and VIII in the presence of its cofactor protein S[22-25]. At the level of factor Xa and thrombin, coagulation activation is inhibited by antithrombin III (AT III), which neutralizes factor Xa and thrombin[26].

▲THE FIBRINOLYTIC SYSTEM

The initiating event in the process of fibrinolysis is the conversion of inactive plasminogen into the active protease plasmin, which is able to cleave cross-linked fibrin[27]. The conversion of plasminogen into plasmin is mediated by plasminogen activators, of which tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) are the most important (Figure 2). tPA is a glycoprotein composed of a single polypeptide chain (sctPA)[28]. In the presence of plasmin, or tissue kallikrein, sctPA is converted into two-chain tPA (tctPA)[29]. Although there are several functional differences between sctPA and tctPA, both activate plasminogen equally well in the presence of fibrin. The main source of tPA is the endothelium. Release of tPA from the endothelial cells can be induced by physical exercise, mental stress, venous occlusion, and a number of substances, such as epinephrine, nicotine amid, histamine, bradykinin, TNF, endotoxin, thrombin, and vasopressin[30-32]. The mechanism of tPA release, induced by these stimuli, is poorly understood. A direct action on these cells has never been found. Hypoxia and acidosis result in a significant increase in tPA[33]. These findings suggest that release of tPA is triggered in areas of ischaemia.
tPA has a high affinity for fibrin and fibrin enhances the affinity of tPA for its substrate plasminogen by the formation of a tPA-fibrin-plasminogen tertiary complex[34]. This implies that tPA has the highest activity where it is required, i.e. locally in the presence of

fibrin, while systemic activation is prevented[35].

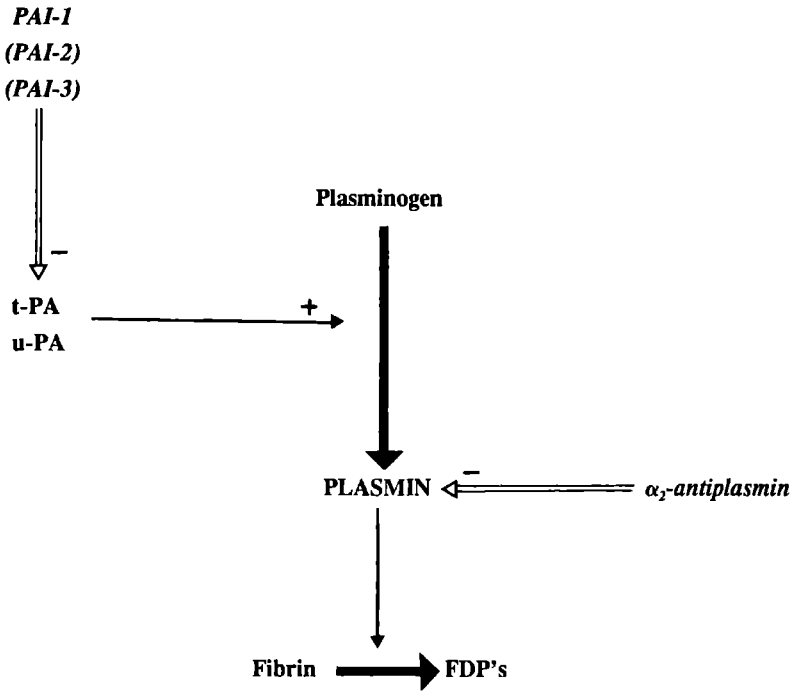

Figure 2. The fibrinolytic system.

uPA circulates in the plasma in a single (pro-urokinase) and in a two-chain form (urokinase) and is present in high concentration in urine[36]. Pro-urokinase behaves like an enzyme itself and is able to directly activate plasminogen[37]. However, its activity is strongly enhanced upon conversion into urokinase by plasmin and kallikrein. uPA is produced by a variety of cells, such as endothelial cells, epithelial cells, macrophages, and various tumor cells[38-41]. Mesothelial cells probably do not release uPA[42]. In contrast to tPA, which is believed to prevent extension of intravascular thrombi, uPA plays a role in extracellular proteolytic mechanisms: increased vascular permeability, cellular migration, tumor invasion and tissue remodelling[41]. uPA does not form a tertiary complex with fibrin and plasminogen and the relative fibrin specificity of uPA is solely dependent on the specific binding of plasminogen to fibrin[43].

Inhibition of fibrinolysis is mediated by plasminogen activator inhibitors and a series

of plasmin inhibitors[44]. Plasminogen activator inhibitor 1 (PAI-1) is the most important inhibitor of tPA and uPA[45]. PAI-1 is mainly present in plasma, endothelial cells and the alpha granules of platelets[46,47]. It is also produced by stimulated mesothelial cells, hepatocytes, melanoma cells and human vascular smooth muscle cells[48]. PAI-1 acts as a pseudosubstrate for tPA or uPA, forming inactive tPA/PAI or uPA/PAI complexes, respectively[49]. It is also capable to inhibit thrombin, which indicates a modulating role in coagulation as well[50,51]. Production and release of PAI-1 are enhanced by several stimuli, such as endotoxin, TNF, interleukin(IL)-1 and corticosteroids[48,52]. Increased levels of PAI-1 have been found in inflammatory processes[53-55].
Two other plasminogen activator inhibitors have been identified: PAI-2 and PAI-3. PAI-2, an inhibitor of both tPA and uPA, was originally isolated from the human placenta and is detectable in plasma in the last trimester of pregnancy[56]. PAI-2 probably is released by human leukocytes, monocytes and macrophages and has recently been identified in inflamed peritoneum[57]. Human omental mesothelial cells also produce PAI-2 upon stimulation with TNF in vitro[48]. PAI-3 is a glycoprotein that appeared to be identical to protein C inhibitor, and inhibits both plasminogen activators and activated protein C. Its exact role in fibrinolysis and coagulation remains to be clarified[58].
Of the protease inhibitors that inhibit plasmin, α_2-antiplasmin is the most prominent, because it has a high affinity for plasmin and is fast acting[59]. During the coagulation process, α_2-antiplasmin is incorporated in the fibrin clot by thrombin activated factor XIII (factor XIIIa), thereby preventing premature lysis of the clot[60]. Beside plasmin, α_2-antiplasmin inhibits factor XIIa, kallikrein, factor XIa and thrombin. However, these actions appear to be of minor importance[61].
Other protease inhibitors, such as α_2-macroglobulin and α_1-antitrypsin are relatively slow inactivators of plasmin, kallikrein, tPA and uPA.

Intra-abdominal and intravascular coagulation are probably similar processes. Various coagulation factors and their natural inhibitors have been found in the peritoneal fluid under normal and pathological conditions, such as peritoneal dialysis, pelvic endometriosis and intra-abdominal infection[62-68].
Knowledge of intra-abdominal fibrinolysis is mainly derived from studies on intra-abdominal adhesion formation[3,69,70].
Under normal circumstances, there is a balance between coagulation and fibrinolysis

in the abdominal cavity If coagulation is activated, fibrin is formed followed by fibrin clot retraction, clot stabilization, and clot destabilization Before retraction, the fibrin clot is susceptible to lysis by tPA, which influences the extent of clot formation The fibrin clot stabilizes as a result of fibrin cross-linking, incorporation of α_2-antiplasmin and local release of PAI-1 After these processes, the fibrin deposit is subject to destabilization, which is mainly caused by inactivation of plasminogen activator inhibitors and plasmin inhibitors At this phase neutrophils invade the clot and promote fibrinolysis by inactivation of α_2-antiplasmin through their product elastase Disruption of fibrin deposits is further enhanced by macrophages During the destabilization phase, tPA may become active again and lyses the fibrin clot The process of fibrinolysis is damped, when the stimulative effect of fibrin on tPA is lost, tPA and uPA are blocked by PAI and plasmin is inactivated by α_2-antiplasmin

▲INTRAABDOMINAL COAGULATION AND FIBRINOLYSIS DURING PERITONITIS

During peritonitis, coagulation most likely is activated by release of TF (procoagulant activity) in the abdominal cavity[62 71 72] TF is expressed by several peritoneal cells, such as mesothelial cells, (submesothelial) endothelial cells and peritoneal macrophages[4 73-76] Cellular expression of TF might be due to cell damage or triggering of intact cells by inflammatory stimuli and bacteria From studies on mesothelial and endothelial cells seeded on vascular prostheses, it is known that the slightest cell injury (detachments of cells during culturing) causes TF expression[76] Macrophages, stimulated in vitro by endotoxin, *Eschenchia coli* and *Bacteroides fragilis,* express TF activity[75 77] However, inhibition of TF activity due to short-chain fatty acids, produced by anaerobic bacteria, has also been demonstrated[78]

Most studies on peritonitis and fibrinolysis have been focused on intra-abdominal adhesion formation and were directed to the peritoneal fibrinolytic activity, assuming that fibrinolysis in the abdominal cavity is controlled by activators and inhibitors present in the peritoneum[72 79 80] These studies almost consistently demonstrated that peritoneal fibrinolytic activity is suppressed by infection[80 81] Hau et al demonstrated a marked decrease of peritoneal fibrinolytic activity in dogs with bacterial peritonitis, which was attributed to a decreased tPA activity[72] A decrease in tPA, but an increase in uPA has been demonstrated immunohistochemically in biopsies of inflamed appendices[42]

In peritoneal biopsies of patients with appendicitis a reduction of fibrinolytic activity was found, which was attributed to an increase of PAI-1[55]. More recently an inhibitory role of PAI-2 in peritonitis has been reported[57].
Reduction of fibrinolytic activity is not a unique feature of infection, since it has been found after mechanical, chemical and ischaemic injury to the peritoneum[80]. A common response to these types of injury is the synthesis and release of cytokines by inflammatory cells[82]. In vitro studies have shown that IL-1 and TNF increase the release of PAI and uPA by endothelial cells, whereas tPA is unaffected or decreased[52,83]. In cultures of human omental mesothelial cells, TNF induces a decrease of fibrinolytic activity as a result of a decrease of tPA and an increase of PAI[48]. In women with pelvic inflammatory disease, an increase of tPA, uPA, and PAI antigens has been demonstrated in the peritoneal fluid[84]. Signs of consumption of plasminogen and antiplasmins, and formation of plasmin-antiplasmin complexes have been found in the peritoneal fluid of both patients with chemical and bacterial peritonitis[68].
All these studies provide evidence that during infection, intra-abdominal coagulation is enhanced and fibrinolytic activity in the surrounding peritoneal tissue is depressed, which is held responsible for persistence of fibrin deposits in the abdominal cavity.

▲MODULATION OF INTRAABDOMINAL COAGULATION OR FIBRINOLYSIS

Potential strategies to prevent intra-abdominal fibrin formation include inhibition of intra-abdominal coagulation and stimulation of fibrinolysis. The anticoagulant heparin, administered both systemically and intraperitoneally, has been studied extensively in experimental peritonitis[85-88]. Hau et al. have shown that heparin, given intraperitoneally, is highly effective in the treatment of lethal peritonitis in dogs[85]. In rats with peritonitis, heparin, administered either subcutaneously or intraperitoneally, significantly prolonged survival time, and reduced the development of adhesions and abscesses in the peritoneal cavity[86 88]. In rabbits with peritonitis after appendiceal ligation, heparin, administered intraperitoneally, decreased mortality, whereas heparin, given subcutaneously, did not[87]. In these experiments, heparin did not influence intrinsic fibrinolytic activity of the peritoneum, although other studies have demonstrated that heparin can augment plasminogen activator induced fibrinolysis[89,90]. The success of heparin was attributed

to reduction of intraperitoneal fibrin formation. However, success has also been attributed to the systemic effects of heparin, such as prevention of intravascular coagulation, promotion of bacterial clearance by preventing the thrombosis of subperitoneal lymphatics, or the observed beneficial effect of heparin on renal function during intraperitoneal sepsis[88 91]. A serious drawback of the administration of heparin is haemorrhage, which has lessened the enthusiasm to use this anticoagulant in clinical practice. So far, no clinical data have been reported on the use of heparin in peritonitis.

More recent experimental studies have focused on prevention of fibrin deposition and intra-abdominal abscesses by plasminogen activators[92-95]. In a rat model of peritonitis, using an infected human fibrin clot, tPA, given intraperitoneally simultaneously with the inoculum, appeared to be highly effective in preventing intra-abdominal abscess formation[93]. Delayed administration of tPA had no effect on the abscess rate, but did reduce abscess size[94]. In this model, early mortality associated with the use of tPA was observed. This mortality was attributed to early bacteraemia[93]. Increased bleeding related to tPA was not found

Other plasminogen activators, such as urokinase and streptokinase, have only been studied in the prevention of adhesions in animal experiments, with conflicting results[96 97]. Clinical data on the use of plasminogen activators in peritonitis are not available.

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CHAPTER 6

COMPLICATIONS OF PLANNED RELAPAROTOMY IN PATIENTS WITH SEVERE GENERALIZED PERITONITIS

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▲INTRODUCTION

"Planned relaparotomy" has become an established surgical treatment modality to manage patients with generalized peritonitis. Several authors have obtained favourable results with this approach, reporting decreased mortality and a low incidence of residual abscesses [1-4]. Furthermore, it was emphasized that "planned relaparotomy" gives the opportunity to early diagnose and treat complications like bowel perforation [3]. However, in our experience this approach has a marked morbidity, to which little attention has been paid in literature [5,6].

Therefore, we analyzed the results of "planned relaparotomy" in patients with the most severe forms of peritonitis: faecal peritonitis due to colonic perforation and postoperative anastomotic leakage, paying special attention to abdominal complications. In addition, we made an attempt to define when to stop planned relaparotomies.

▲PATIENTS AND METHODS

From 1989 to 1993, 24 consecutive patients underwent "planned relaparotomy" for massive faecal peritonitis due to large bowel perforation (n=15) or postoperative anastomotic leakage (n=9) in our surgical unit. There were 18 men and 6 women, their mean age was 63 years, ranging from 31 to 89 years. Eleven patients were referred from other hospitals.

All patients were admitted at the surgical intensive care unit (ICU). Tobramycin and Metronidazole were given as initial antibiotic therapy. If cultures were available, antibiotics were adjusted to these. At the first laparotomy management consisted of elimination of the source of peritonitis, debridement and intra-operative lavage. The abdomen was "closed" with a zipper (Ethizip®), attached to the fascial edges. Planned relaparotomies were performed every 24 to 48 hours. The abdomen was debrided, removing fibrinous deposits and septic collections, samples of which were cultured. Finally the abdominal cavity was rinsed thoroughly with warm normal saline solution. Planned relaparotomies were discontinued when the abdomen was judged to be 'clean' and sepsis was subsided. The abdomen was closed whenever possible, preferably without the use of foreign material. One author (R.P.B.) personally participated in the surgical management of all patients.

The following data were extracted from the medical records: number of planned relaparotomies, number and indication for emergency relaparotomy, duration of ICU stay, results of cultures taken from the abdominal cavity, (causes of) death during hospital stay, time and type of abdominal wall closure, and number and type of abdominal complications (intra-abdominal abscess, intra-abdominal haemorrhage for which emergency relaparotomy was needed, gastrointestinal haemorrhage, bowel perforation, enterocutaneous fistula, anastomotic dehiscence and stomal necrosis).
At the day of admittance a modified APACHE II score (no assessment of Glascow Coma Scale) was determined for each patient[7]. During the first 7 days and subsequently once a week a modified multiple organ failure (MOF) score (no assessment of central nervous system failure) was calculated[8]. These modifications were necessary because a reliable assessment of the neurological state could not be made retrospectively.
The samples taken from the abdominal cavity were cultured for both aerobes and anaerobes according to standard procedures. Growth was determined semiquantitatively and expressed in colony forming units (cfu)/ml.
The statistical tests used are given within the text. When $p<0.05$, a difference was considered significant.

▲RESULTS

A total of 136 planned relaparotomies and 23 emergency relaparotomies were performed in 24 patients during a median ICU stay of 29.5 days, ranging from 9 to 154 days. Mean ± SD modified APACHE II score was 18.7 ± 5.6. There was a median number of 5 planned relaparotomies per patient, ranging from 2 to 13. All emergency relaparotomies were performed for haemorrhage.

Mortality

Seven of 24 patients (29%) died during hospital stay. All these patients were referred from other hospitals. Five patients died as a result of a multiple organ dysfunction syndrome, one due to an intracerebral bleeding three weeks after the first operation, and in one patient treatment was stopped because of disseminated colon cancer. In Figure 1 the relation between the modified MOF score and survival is shown. From

day 1 on, the score of nonsurvivors was significantly higher than that of survivors. MOF score of survivors remained unaltered during the first seven days and decreased thereafter ($p<0.01$; Pitman test), whereas that of nonsurvivors did not change.

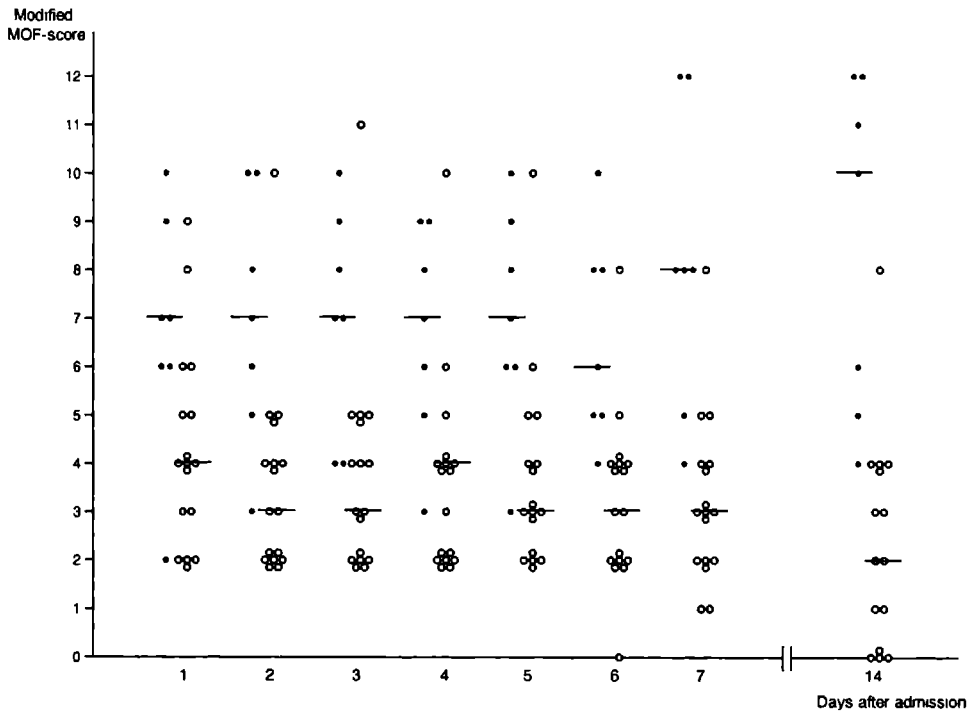

Figure 1 Relation between the modified MOF score and survival Scores of nonsurvivors (●, n=7) were significantly higher ($p<0.05$, Yates and Cochran test) than those of survivors (○, n=17) at all determination points Bars represent median values

Morbidity

Major complications were observed in all seven patients who died and in 10 (59%) of the 17 surviving patients. The number of complications in nonsurvivors (24 in 7)

was significantly higher ($p<0.02$, Yates and Cochran) than that in survivors (25 in 17)
A residual intra-abdominal abscess was found in only one patient This abscess was drained percutaneously three months after cessation of "planned relaparotomy"
Intra-abdominal haemorrhage occurred 23 times in 14 patients 14 times in nine of the 17 surviving patients and nine times in five of the seven nonsurvivors In most cases gauze packing had to be carried out In one patient an iliac artery was ligated because of a bleeding mycotic aneurysm
Six episodes of upper gastrointestinal haemorrhage were documented in five patients of whom two died All bleedings were treated conservatively
Six small bowel perforations were diagnosed in five patients during re-explorations In three of these patients an enterostomy was created, of whom in two the enterostomies became necrotic and had to be corrected In two patients the bowel was anastomosed primarily, no complications of the primary anastomoses were observed
An end-enterostomy was created in 20 patients at initial surgery and in three of them additional enterostomies were made in the course of the planned relaparotomies Six of these 20 patients (30%) needed surgical correction of their enterostomy because of necrosis and/or perforation Four times in three patients, a small bowel enterostomy had to be corrected, three times a colostomy Three patients with necrosis of an enterostomy died
Six small bowel fistulas occurred in four patients In one patient, definitive treatment of the fistula was carried out after three months without further complications Three patients died with persisting small bowel fistulas
There was a strong correlation between the number of complications and the number of planned relaparotomies ($R=0.90$, $p<0.001$, Spearman) (Figure 2) All patients, who underwent more than five planned relaparotomies, had complications
Patients with complications stayed significantly longer ($p<0.05$, Wilcoxon) in the ICU than patients without complications [median (range) 36(14-154)days *versus* 14(9-37)days]

Cultures

At initial laparotomy the cultures of the abdominal samples revealed more than 10^3 cfu/ml in all 24 patients In 96% of the patients a polymicrobial flora was found In 21 patients the abdomen became "clean" -i e $< 10^3$ cfu/ml- after a median number

of 3 relaparotomies (range, 1 to 9). The remaining three patients developed a tertiary peritonitis, two with *Candida albicans*, one with *Pseudomonas aeriginosa*. At autopsy, four patients had a "clean" abdomen, and three a tertiary peritonitis. The abdominal cavity of all 17 surviving patients became "clean". Two of these 17 patients were successfully treated for a *Candida* peritonitis during their ICU stay.

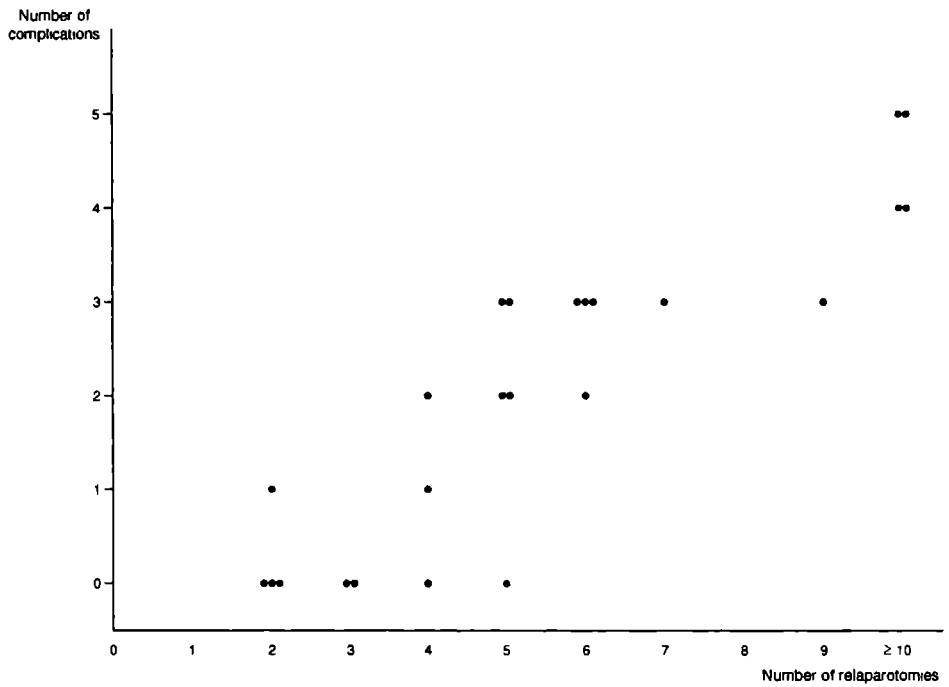

Figure 2 Correlation between the number of intra-abdominal complications and the number of relaparotomies ● patients (n=24) R=0 90, p<0 001, Spearman's rank test

Abdominal wall defects

In 13 patients (11 survivors, 2 nonsurvivors) the abdomen was closed on an average of 14.2 days (range, 5 to 25 days) after admission. Patients in whom fascial closure was achieved had undergone a median (range) number of 3 (2-5) relaparotomies,

which is significantly less ($p<0.05$; Wilcoxon) than that in patients without closure; 8 (5-13).

▲DISCUSSION

"Planned relaparotomy" has gained wide acceptance among surgeons as the treatment of choice for patients with severe forms of peritonitis. However, the indications for this treatment are still debatable. The mortality of patients treated by "planned relaparotomy" varies between 7 and 67%[1,9]. Most likely this wide variation is a result of patient selection. Some surgeons, reporting a low mortality, use this method liberally including patients with acute appendicitis and perforated duodenal ulcers, whereas others are more restrictive[3,6,10,11]. Therefore, we have selected those patients in whom the indication for "planned relaparotomy" will be accepted by every surgeon who applies this method: diffuse suppurative peritonitis due to perforation of the colon or anastomotic dehiscence, in which the abdominal cavity remains grossly contaminated after the initial laparotomy. Control of the source of contamination is the main goal in patients with generalized peritonitis. We reached this goal in all our patients at the initial laparotomy by creating an enterostomy, in the majority of cases. However, necrosis of enterostomies occurred frequently, which was mainly due to difficulties mobilizing the oedematous bowel with its shortened and thickened mesentery. This complication is prevented when a loop-end enterostomy is created in stead of an end-enterostomy (Figure 3). Primary anastomosis of the bowel might be another good alternative[12].

"Planned relaparotomy" is performed to control infection, to reverse the systemic consequences of peritonitis, i.e. multiple organ dysfunction, and to diagnose complications in an early stage. Infection was controlled effectively since a residual abscess was found in only one patient. Moreover, cultures from the abdominal cavity of 21 patients revealed less than 10^3 cfu/ml in 62% after two, in 76% after three, and in 95% of them after four relaparotomies. It is obvious that more relaparotomies were performed than necessary for control of infection. In most patients planned relaparotomies were continued because of the persistent septic state of the patients. However, in retrospect, "planned relaparotomy" had no detectable beneficial influence on organ function.

Haemorrhage, bowel perforation and bowel fistula were frequent complications, encountered particularly after more than four relaparotomies, indicating that multiple planned

relaparotomies are not harmless. The risk of bleeding and damage to the bowel increases in time because firm granulation tissue replaces the initially present loose fibrinous serosal attachments. In concert with a decrease of collagen content, the bowel wall becomes very vulnerable and perforates following traction, particularly in an "open" abdomen[13].

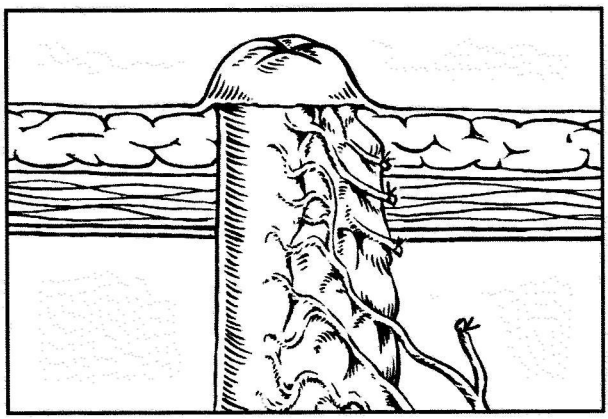

Figure 3 A: End-enterostomy. Because of the oedematous abdominal wall and mesentery, the necessary dissection to create an end-enterostomy easily jeopardizes its vascularization, especially in circumstances of splanchnic vasoconstriction.

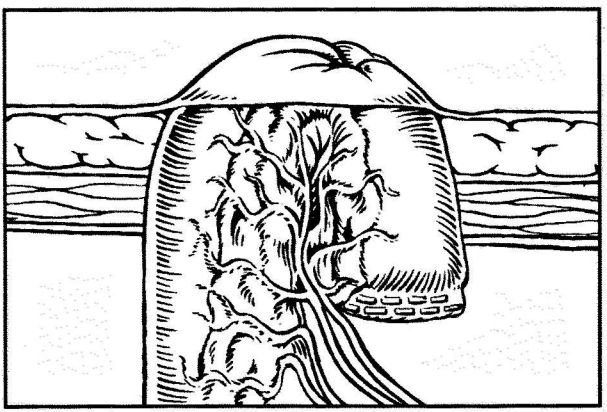

Figure 3 B: Loop-enterostomy. Mesenterial dissection is not necessary, leaving vascularization intact.

We, like many authors, used criteria such as a "clean abdomen" or "signs of sepsis subsided" for cessation of planned relaparotomies[3 6 10]. These criteria are not well defined and carry the risk of performing too many re-explorations, because sepsis may persist, whereas intra-abdominal infection is controlled.

Based on this study we suggest to use cultures obtained at the time of operation to guide the decision for further re-explorations. The severity of organ dysfunction does not play a role in this decision.

The - $< 10^3$ cfu/ml - criteria for cessation of planned relaparotomies needs to be confirmed. When we had used this criteria, less planned re-explorations had been performed, intra-abdominal infection control had been as effective, less intra-abdominal complications might have occurred, and in more patients the abdomen could have been closed after cessation of planned relaparotomies.

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CHAPTER 7

EARLY AND LONG TERM RESULTS OF NECROSECTOMY AND PLANNED REEXPLORATIONS FOR INFECTED PANCREATIC NECROSIS

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▲INTRODUCTION

The treatment of patients with acute pancreatitis is primarily conservative, as acute pancreatitis responds well to supportive treatment in the majority of patients. 15-20% of the patients has severe forms of acute pancreatitis with organ failure and/or local complications, who may need surgical treatment[1-3]. Infected (peri)pancreatic necrosis, identified by computed tomography (CT)-guided fine needle aspiration biopsy, is the main indication for surgical intervention[1,4,5], deterioration of the condition of the patient despite supportive therapy in the intensive care unit (ICU) might be another[6].
Several surgical approaches such as total or partial pancreatic resection, necrosectomy followed by closed drainage or closed continuous lavage, and partial necrosectomy followed by planned re-explorations with "open" drainage have been advocated to deal with (peri)pancreatic necrosis[7-11].
Pancreatic resection should be avoided because of high mortality and morbidity and the risk of diabetes mellitus as a result of removal of viable pancreatic tissue due to overestimation of the amount of necrosis[7,12].
In our previous experience with closed drainage after necrosectomy, half of the patients died of sepsis and multiple organ dysfunction syndrome (MODS) and 26% had residual abscesses due to insufficient drainage[8].
Necrosectomy followed by closed continuous lavage, as strongly recommended by the group of Beger, results in low mortality[3]. However, the reoperation rate due to recurrent intra-abdominal sepsis is high.
Planned re-explorations after initially limited necrosectomy offer the opportunity to repeatedly remove necrosis and infected material. Several authors have reported a low residual abscess rate and decreased mortality with this treatment modality[1,10,11,13].
Since 1987 we have treated patients with infected pancreatic necrosis by limited necrosectomy, planned re-explorations and "open" drainage. It was the aim of this study to evaluate the early and long term results of this surgical approach.

▲MATERIALS AND METHODS

From 1987 to 1990 10 patients with infected (peri)pancreatic necrosis, as diagnosed

by CT scan and fine needle aspiration, were treated by limited necrosectomy, planned re-explorations and "open" drainage. All patients had extensive (peri)pancreatic necrosis, classified as Balthazar D and E, and suffered from MODS[14]. Twenty-five patients with necrotizing pancreatitis, who were treated in the same period, were excluded from this study because they had sterile pancreatic necrosis or a (late) pancreatic abscess. Access to the peritoneal cavity was obtained via a supra-umbilical transverse laparotomy and the lesser sac was opened by dividing the gastrocolic ligament. A limited necrosectomy of the (peri)pancreatic area was performed. The abdomen was rinsed with several litres of normal saline solution and the lesser sac was packed with gauzes. Seven patients received a needle catheter jejunostomy for early enteral feeding.
Re-explorations were done at least once a day in the operation theatre under general anaesthesia. Fluid collections and sequestrated necrotic material were removed. When necrosis was replaced by granulation tissue and the inflammatory process had become localized, re-explorations were performed in the ICU under sedative medication every two days. Explorations were discontinued as the lesser sac had been obliterated. In none of the patients the fascia could be closed.
All patients were treated in the ICU by mechanical ventilation, aggressive fluid resuscitation, use of vasopressors, systemic antibiotics and selective bowel decontamination[15]. Five patients needed dialysis due to renal insufficiency. Severity of illness was scored following the Ranson criteria[16] at admission, and the APACHE II score at the day of surgery. Hospital mortality and morbidity were carefully assessed. Special attention was paid to complications associated with the surgical technique.

Surviving patients were examined at least three years after discharge. Long term morbidity and return to work or former activities were assessed and patients underwent tests to evaluate their pancreatic exocrine and endocrine function. Informed consent to perform the pancreatic function tests was given by patients and healthy volunteers.

Pancreatic exocrine function
To determine exocrine function, faecal fat excretion was measured on three consecutive days with a standard meal, consisting of 70 g/day fat. An average fat excretion lower than 7 g/day was considered normal. Faecal fat was measured using a standard method[17].

Pancreatic endocrine function

An intravenous glucose tolerance test (IVGTT) was performed to assess pancreatic endocrine function. After intravenous bolus injection of 25 g/kg glucose, peripheral venous blood samples were taken at -15, 0, 5, 10, 20, 30, 40, 50 and 60 minutes for determination of glucose, insulin and glucagon levels. For comparison, an IVGTT was performed in healthy volunteers, who were matched for age, sex and body weight.

Assays

Blood glucose was measured in duplicate using the hexokinase method.
Insulin and glucagon were determined by radio-immunoassay[18-20].

Statistics

Mean values and standard error of the mean (SEM) of glucose, insulin, and glucagon levels are given. Statistical analysis of these parameters was performed, using ANOVA with Duncan's method to correct for multiple comparisons. Glucagon/insulin ratios were analyzed after log-transformation. A p-value less than 0.05 was considered significant.

▲RESULTS

The characteristics of the patients are presented in Table 1. There were 6 men and 4 women. Their mean age was 51.3 years (range, 29-81 years). The median number of Ranson criteria was 6 (range, 5-8). The median APACHE II score was 19 (range, 12-25). The median number of re-explorations in the operation theatre was 13 (range, 7-28); for survivors 9 (range 7-15) and for nonsurvivors 25 (range, 17-28).
The median duration of ICU stay was 29 days (range, 21-76 days); for survivors 28 days (range, 21-38 days) and for nonsurvivors 46 days (range, 25-76 days).
Cultures of the pancreatic necrotic tissue revealed gram-negative bacteria in one patient, gram-positive bacteria in four patients, and both gram-negative and gram-positive bacteria in the remaining five patients. In one patient *Candida albicans* was isolated (Table 1).

Table 1 Characteristics of 10 patients with infected pancreatic necrosis

Patient No	Sex	Age (yrs)	Aetiology of pancreatitis	No of Ranson criteria	APACHE II score	Cultures	Number of re-explorations in theatre	Major complications	Outcome	Return to work or former activities
1	♂	55	alcohol abuse	6	20	CNS *Enterococci*	8	–	recovered	yes
2	♀	79	gallstones	8	24	*E coli*	17	bleeding lesser sac colonic necrosis	died	–
3	♀	65	unknown	7	15	*Streptococci* *E coli* anaerobes	8	–	recovered	yes
4	♂	49	ischaemic (cardiac surgery)	5	25	*Enterococci* *E coli*	25	bleeding lesser sac jejunal fistula	died	–
5	♂	81	gallstones	5	19	CNS	9	–	recovered	yes
6	♂	29	alcohol abuse	8	18	*Enterococci* *Candida*	11	–	recovered	no
7	♂	39	alcohol abuse	7	21	*Enterococci* CNS	28	bleeding lesser sac colonic necrosis jejunal fistula necrosis jejunostomy necrosis ileostomy	died	–
8	♀	41	steroids (kidney transplant)	6	17	*Enterococci* CNS	15	bleeding pelvis	recovered	yes
9	♀	34	trauma	5	12	*E coli* *Enterococci* *St aureus*	7	bleeding lesser sac colonic fistula duodenal fistula necrosis jejunostomy	recovered	yes
10	♂	41	alcohol abuse	6	19	*E coli* *St aureus*	15	–	recovered	no

E coli, *Eschenchia coli*; CNS, *coagulase negative staphylococcus*; *St aureus*, *staphylococcus aureus*

Early results

Three patients died due to MODS, seven patients survived. No patient who died, had a septic focus in the abdomen at the time of death. At autopsy, the pancreas was largely intact in two patients, and one patient had only a few necrotic spots in the pancreas.
None of the surviving patients developed a residual intra-abdominal abscess. During re-explorations 14 major intra-abdominal complications were encountered in five patients, of whom three died.
Four patients had a bleeding from the lesser sac and one a bleeding from the pelvis, needing surgical reintervention and bloodtransfusions.
Two patients developed necrosis of the transverse colon and were treated by partial colectomy and diverting ileostomy.
In three patients, four enterocutaneous fistulas developed: two jejunal, one duodenal, and one colonic. The jejunal fistulas were treated by resection and primary anastomosis. The duodenal and colonic fistulas closed spontaneously. Of a total of four small bowel enterostomies made, three became necrotic and were corrected surgically.
The lesser sac of the surviving patients gradually obliterated during an average period of 50 days (range, 24-80 days). Residual but transient pancreatico-cutaneous fistulas developed in four patients which closed spontaneously within one year.

Long term results

Abdominal wall defects
All surviving patients had a large abdominal wall defect. In four patients, the defects were closed because of discomfort or cosmetic reasons, on a average of 2.5 years (range, 1-4 years) after initial surgery, with the use of a Goretex® patch in three, and a Marlex® patch in one patient. There were no complications associated with these repairs. Three patients declined surgical correction.

Pancreatic exocrine function
In six patients, clinically without steatorrhoea, the daily fat excretion was normal (median 3.3, range 0.65-6.3 g/day). One patient (no. 3) had an excretion of more than 10 g/day

and needed medication to correct steatorrhoea.

Pancreatic endocrine function

One patient (no. 8) had developed permanent insulin dependent diabetes mellitus. The results of the IVGTT of the other patients and the healthy volunteers are presented in Figure 1, 2, 3, and 4. In the basal state blood glucose and plasma insulin levels were similar in both groups, whereas plasma glucagon levels and glucagon/insulin ratios of the patients were significantly higher ($p<0.05$) compared with healthy controls. After glucose loading, glucose tolerance as measured by the K value[21] was impaired in all patients and insulin and glucagon levels were significantly higher ($p<0.05$) than in controls.

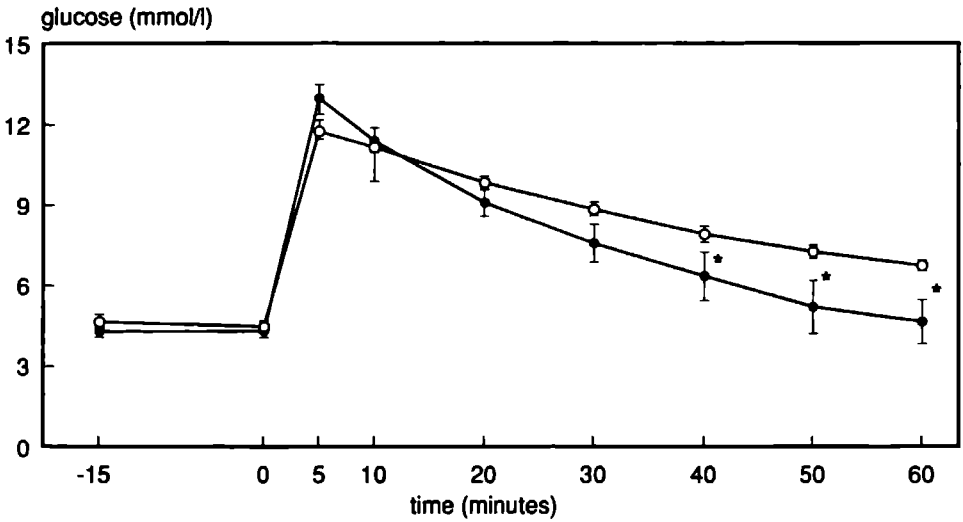

Figure 1 Blood glucose level during intravenous glucose tolerance test (IVGTT) of patients (○) and healthy controls (●) Median values and SEM are given At 40, 50 and 60 minutes after bolus injection differences are significant ($p<0.05$) K-values of patients were 0.8, 0.8, 0.9, 0.9, 0.9, and 1.1 K-values of controls were 1.3, 1.4, 2.3, 2.6, 2.7, and 2.9 (K-values > 1.1 are normal)

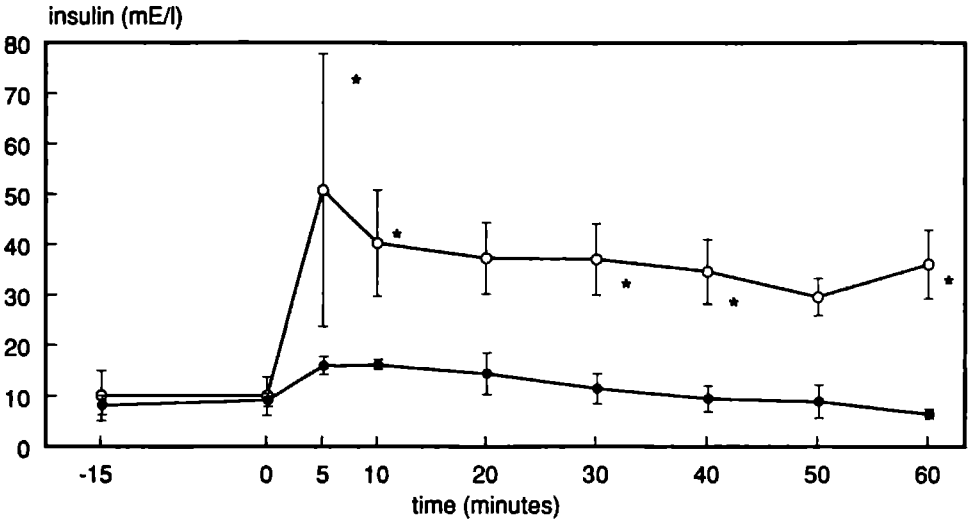

Figure 2. Insulin level during IVGTT of patients (O) and healthy controls (●) Median values and SEM are given. At 5, 10, 30, 40 and 60 minutes after bolus injection differences are significant ($p<0.05$).

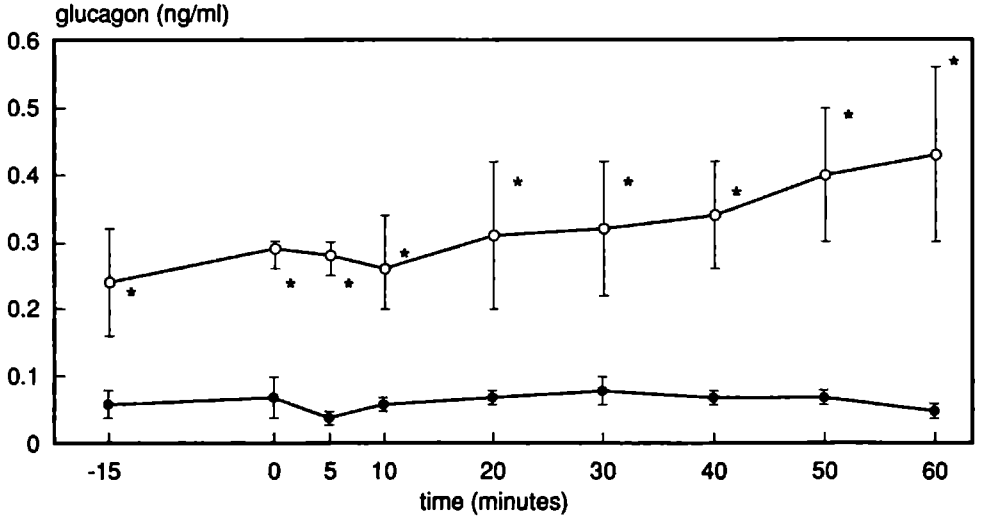

Figure 3. Glucagon level during IVGTT of patients (O) and healthy controls (●). Median values and SEM are given. From 0 to 60 minutes after bolus injection differences are significant ($p<0.05$).

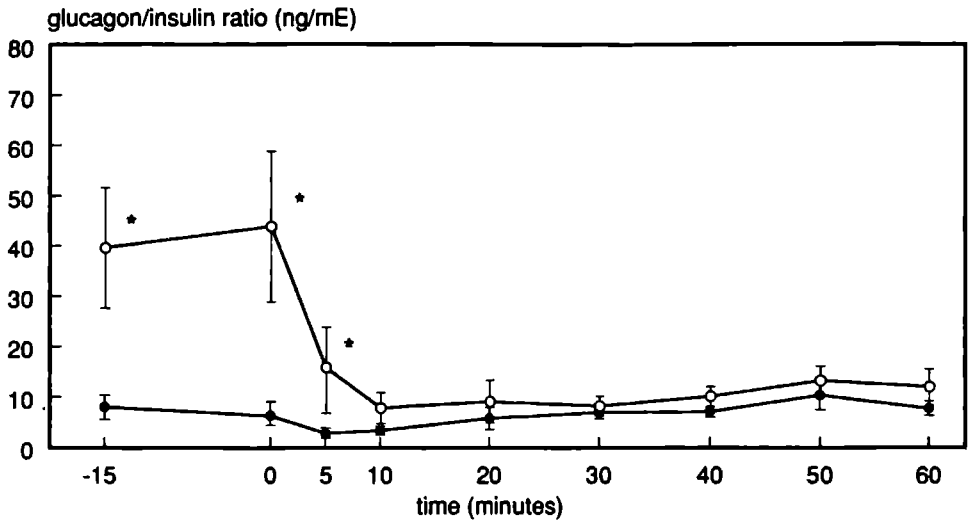

Figure 4 Glucagon/insulin ratio during IVGTT of patients (○) and healthy controls (●) Median values and SEM are given Baseline ratios and ratios at 5 minutes after bolus injection differ significantly ($p<0.05$)

▲DISCUSSION

The present study demonstrates that management of infected pancreatic necrosis by limited necrosectomy and planned re-explorations is effective in controlling the local problem of necrosis and infection. However, this technique is associated with a high complication rate.

Repeated limited necrosectomy and packing of the lesser sac are advised as the best treatment modality for extensive infected pancreatic necrosis minimizing the incidence of recurrent intra-abdominal sepsis and improving patient survival[9,10,13]. The favourable results are explained by the fact that this method directly addresses the problem of continuing inflammation and progress of retroperitoneal necrosis, and provides optimal drainage. Using other drainage techniques e.g. sump drainage and continuous closed lavage, reoperation rate is high due to recurrent intra-abdominal sepsis[3]. These latter techniques seem particularly successful when necrosis is less extensive and confined

to the lesser sac.

A significant number of serious local complications as intra-abdominal haemorrhage, intestinal necrosis and fistulas, was found in our series. Despite limited necrosectomy, haemorrhage is a significant problem, associated with increased morbidity and mortality[2,10,11]. In our series, haemorrhage occurred particularly in the initial phase of the treatment due to clotting disturbances, involvement of the splenic and middle colic vessels in the necrotic process, and gauze packing. The latter factors are also responsible for the occurrence of colonic necrosis and gastrointestinal fistulas[2,10,11]. The use of non-adherent gauzes, transverse incisions, and avoidance of a prolonged "open abdomen" might be measures minimizing the occurrence of fistulas[10,11,22]. These local complications are not exclusively linked with this techniques, since the same are reported in association with postoperative closed continuous lavage[23]. The high incidence of necrosis of an enterostomy is mainly due to the difficulty mobilizing the bowels because of oedema of the bowel wall and mesentery. Moreover, the risk of necrosis of enterostomy is enhanced by septic shock and the use of vasopressors. This complication might be avoided using loop-end enterostomies or performing primary anastomoses, whenever possible.

Mortality in our group of patients was associated with a high number of re-explorations, a high number of complications, and a long duration of ICU stay. It was of interest that all three deaths had pre-existent morbid obesity, another factor that has been associated with increased mortality[24].

Data on pancreatic function after recovery from acute necrotizing pancreatitis are scarce. The majority of available data refers to function after resectional therapy, which is accompanied by a high incidence of diabetes[7,12]. Büchler et al. demonstrated long term exocrine and endocrine functional impairment in about two-third of their patients, treated with necrosectomy, followed by continuous postoperative lavage of the lesser sac[25].

Extensive pancreatic necrosis inevitably will lead to pancreatic exocrine and endocrine insufficiency. However, both in our series as in others the incidence of steatorrhoea and diabetes is low[10,11]. These results might not only be explained by a large overcapacity of pancreatic tissue, but by the fact that only part of the patients with necrotizing pancreatitis has significant parenchymal necrosis as shown histologically in resected pancreatic specimens, at autopsy in our patients, and as demonstrated by normal

endoscopic retrograde pancreatograms after recovery from necrotizing pancreatitis[26,27]. Although most of our patients had no diabetes, they had an impaired glucose tolerance. Surprisingly, this was not caused by lowered serum insulin levels. On the contrary, insulin levels were significantly higher than in healthy controls.

The impaired glucose tolerance might be caused by peripheral insulin resistance with consequently increased insulin release by the ß-cells of the pancreas. However, this does not explain the elevated glucagon levels in the basal state and after glucose challenge.

Impaired glucose tolerance might be secondary to increased glucagon levels. Particularly in the first minutes after glucose challenge, the higher glucagon/insulin ratio disturbs hepatic glucose metabolism, which may result in hepatic glucose production instead of cessation of hepatic glucose production[28].

We do not have an explanation for the elevated glucagon concentrations. Further studies will be conducted to elucidate the mechanisms involved in the disturbance of glucose tolerance and the elevation of insulin and glucagon levels in patients after treatment for infected pancreatic necrosis.

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CHAPTER 8

COAGULATION AND FIBRINOLYTIC RESPONSES IN HUMAN PERITONEAL FLUID AND PLASMA TO BACTERIAL PERITONITIS

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▲INTRODUCTION

In bacterial peritonitis fibrin is formed in the abdominal cavity, as is established macroscopically at surgery. Activation of the intra-abdominal coagulation system induces the formation of fibrin in the abdominal cavity. Intra-abdominal fibrin plays a key role in the local host response to inflammation, and prevents early bacteraemia and sepsis, by entrapment of bacteria[1]. Fibrin has only a temporary role and must be resolved in order to resume normal tissue function. For the resolution of intra-abdominal fibrin, stimulation of the intra-abdominal fibrinolytic system is required. However, this fibrinolytic system seems to be inhibited during bacterial peritonitis, as has been demonstrated in several experimental studies[2-4]. As a consequence, intra-abdominal fibrin deposits may persist, and these are a nidus of intra-abdominal abscesses[1,5]. In patients with bacterial peritonitis, intra-abdominal abscesses are associated with a marked morbidity and mortality[6,7].
There is no detailed knowledge from clinical studies about fibrin formation and fibrinolysis in peritonitis. Therefore, we conducted a controlled clinical study to determine the effect of bacterial peritonitis on coagulation and fibrinolysis in peritoneal fluid and plasma.

▲PATIENTS AND METHODS

Study design

Twenty-five patients, who underwent emergency laparotomy for peritonitis were studied. Peritonitis was due to perforated appendicitis in 10, duodenal perforation in five, perforated diverticulitis in five, small bowel perforation in two, toxic megacolon with perforation in two, and infected ascites due to pancreatic necrosis in one patient. Abdominal cultures revealed bacterial growth in all patients. There were 16 males and nine females. Their mean age was 48 years, ranging from 11 to 90 years.
Seven patients, who underwent elective cholecystectomy for gallstone disease served as controls. Bacterial cultures of the peritoneal fluid were negative in all control patients. There were three males and four females. Their mean age was 50 years, ranging from 12 to 75 years.

After incision and meticulous haemostasis, 10 ml of peritoneal fluid were aspirated from the abdominal cavity in all patients with peritonitis In cholecystectomy patients, the amount of fluid aspirated varied between 0 5 and 2 ml, and was diluted with normal saline until a total amount of 10 ml

Venous blood (10 ml) was drawn simultaneously with peritoneal fluid aspiration Peritoneal fluid and blood were collected in a citrate-tube (1 10) and a Stabilyte® tube (1 10), respectively, and placed in melting ice[8] Within 30 minutes the samples were centrifuged at 4°C, and the supernatants were stored at -80°C, until assays were performed

Activation of coagulation was assessed by measuring the thrombin-antithrombine III (TAT) complex, as thrombin (generation) is the final step in the coagulation cascade before fibrin formation

To analyze fibrinolysis, tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), plasminogen activator inhibitor (PAI), plasmin-$\alpha_2$antiplasmin (PAP) complex, and fibrin degradation products (FbDP) were assessed

This study was approved by the hospital ethical committee and informed consent was obtained from all patients and/or their parents

Assays

TAT complex was measured by ELISA (Enzygnost TAT, Behringwerke AG, Marburg, Germany)

tPA antigen was measured by ELISA (Asserachrom tPA, Boehringer, Mannheim, Germany) The assay measures both free and complexed (with PAI-1) human tPA and is calibrated against the International Standard for tPA

tPA activity was measured by Coaset-tPA assay (Kabi Diagnostica AB, Mölndal, Sweden) as described by Nilsson et al [9]

PAI-1 antigen was measured by ELISA (Biopool AB, Umea, Sweden) The assay measures free, latent as well as complexed (with tPA or uPA) human PAI-1

PAI activity was measured by Berichrom-PAI assay (Behringwerke AG, Marburg, Germany) as described by Stief et al [10]

uPA antigen in plasma and in peritoneal fluid was measured by ELISA according to Binnema et al [11] Concentrations were expressed in percentage of the uPA concentration of normal pooled plasma of healthy volunteers

PAP complex was measured by ELISA (EIA APP micro, Behringwerke AG, Marburg,

Germany).
FbDP were measured by ELISA (Fibrinostika FbDP, Organon Technika, Turnhout, Belgium).

Statistics

Presented data are expressed as median values and 95% confidence intervals. The Mann Whitney-U test was used for statistical analysis of differences between groups. Correlations between plasma and peritoneal fluid values were analyzed by Spearman's rank test. A p-value less than 0.05 was considered significant.

▲RESULTS

Extensive fibrin deposition was found at laparotomy in all patients with bacterial peritonitis, but in none of the control patients. Activated coagulation in the abdominal cavity of patients with peritonitis was demonstrated by significantly higher TAT complex concentrations, as compared with controls (Table 1).
Stimulation of the fibrinolytic system in the peritoneal fluid of patients with peritonitis was demonstrated by significantly elevated concentrations of tPA, uPA, PAP complex and FbDP, as compared with those concentrations in the peritoneal fluid of controls (Table 1). Median peritoneal fluid concentrations of tPA and uPA in patients with peritonitis were about 65 fold and 10 fold, respectively, higher than those in controls. PAI-1 antigen showed a more than 400 fold increase. Hence, concentrations of PAP complex and FbDP in the peritoneal fluid of patients with peritonitis were significantly elevated, in comparison with those in controls.
By contrast, plasma concentrations of TAT and PAP complexes, and tPA and PAI-1 antigen and activity, were comparable in patients with and without peritonitis. Only plasma concentrations of uPA antigen and FbDP in patients with peritonitis were higher than those in control patients (Table 2).
Neither in patients with peritonitis nor in control patients, the level of any coagulation or fibrinolytic factor, measured in the plasma, correlated with that, measured in the peritoneal fluid.

Table 1 Parameters of coagulation and fibrinolysis in the peritoneal fluid of patients with peritonitis (n=25) and control patients (n=7) Results in control patients were corrected for dilution at sampling Median values (95% confidence intervals) are given

peritoneal fluid	peritonitis	control	p-value
tPA antigen (ng/ml)	66 1 (37 5-180)	1 1 (0 5-1 8)	<0 05
tPA activity (IU/ml)	6 5 (3 7-11 9)	<0 25 (<0 25-0 3)	<0 05
PAI-1 antigen (ng/ml)	395 (147-588)	0 5 (<0 5-1 3)	<0 05
PAI activity (AU/ml)	14 3 (7 4-22 2)	<0 3 (<0 3-<0 3)	<0 05
uPA antigen (%)	740 (168-2000)	78 (14-168)	<0 05
TAT (ng/ml)	5500 (2976-9094)	89 (30-198)	<0 05
PAP (ng/ml)	10952 (4834-15469)	57 (17-123)	<0 05
FbDP (ng/ml)	40360 (6475-200000)	126 (31-463)	<0 05

▲DISCUSSION

This study demonstrates, that in the abdominal cavity of patients with bacterial peritonitis both the coagulation system and the fibrinolytic system are stimulated The resultant fibrin deposition probably reflects a relative deficit of fibrinolytic activity, due to strongly enhanced inhibition of plasminogen activators

The pronounced, elevated concentrations of TAT complex in the peritoneal fluid of

Table 2 Parameters of coagulation and fibrinolysis in the plasma of patients with peritonitis (n=25) and control patients (n=7) Median values (95% confidence intervals) are given

plasma	peritonitis	control	p-value
tPA antigen (ng/ml)	7 9 (4 9-12 6)	6 4 (3 4-10 3)	ns
tPA activity (IU/ml)	2 3 (1 7-3 3)	1 3 (0 9-2 4)	ns
PAI-1 antigen (ng/ml)	15 (10 2-22 3)	13 8 (5 9-39 9)	ns
PAI activity (AU/ml)	1 7 (0 9-2 9)	1 4 (0 8-2 3)	ns
uPA antigen (%)	204 (138-272)	120 (96-172)	<0 05
TAT (ng/ml)	5 2 (3 3-16 5)	2 6 (1 4-5 5)	ns
PAP (ng/ml)	709 (450-917)	381 (290-567)	ns
FbDP (ng/ml)	1201 (559-2867)	350 (193-594)	<0 05

all patients with peritonitis demonstrate intra-abdominal stimulation of coagulation The coagulation cascade may be triggered by expression of tissue factor (procoagulant activity) on injured mesothelial and endothelial cells in the peritoneum[12 14] Procoagulant activity may also originate from peritoneal macrophages, which predominate in the abdominal cavity early in the course of peritonitis, and express tissue factor upon stimulation with, for example, endotoxin and tumor necrosis factor[14 15] The relative contribution of separate cell types to intra-abdominal coagulation has not been clarified yet It seems that the activation of coagulation was confined to the abdominal cavity,

as plasma TAT concentrations in most patients with peritonitis were not elevated. In a few patients with sepsis, high plasma levels of TAT were found, which is in concordance with earlier reports on the relation of sepsis and systemic hypercoagulability [16,17].
Based on studies of fibrinolytic activity of normal and inflamed peritoneum, it is commonly assumed that fibrinolytic activators are reduced in peritonitis [2,18,19]. Our data, on the contrary, show an increase of fibrinolytic activators. This finding is in agreement with that of Dörr et al., who reported increased fibrinolytic activity and fibrin degradation in the peritoneal fluid of women with pelvic inflammatory disease [20]. Increased tPA antigen levels in the peritoneal fluid, associated with normal plasma levels of tPA antigen in the patients with bacterial peritonitis, suggest local production of tPA. In particular, mesothelial cells and submesothelial endothelium might be responsible for the production of tPA [21,22]. The increased tPA concentrations, which we found, might be explained by a prompt release from a preformed storage pool within these cells. Several 'stressfactors' and inflammatory mediators, stimulated upon bacterial infection, may induce such a release of tPA in vivo [23]. However, these findings are not supported by in vitro experiments, in which inflammatory stimuli, like tumor necrosis factor and interleukin-1, inhibited tPA release by cultured mesothelial and endothelial cells [22,24]. As an alternative explanation, increased levels of tPA might be due to leakage from mesothelial cells, which are damaged during peritoneal infection.
PAI-1 forms a "one to one" inactive complex with both tPA and uPA. Since the increase of PAI-1 in the peritoneal fluid of patients with peritonitis was much higher than that of tPA and uPA, complete inhibition of fibrinolytic activity in the abdominal cavity might be expected. However, plasmin activity was still present as shown by elevated levels of PAP complex and FbDP. Since plasminogen activation, mediated by tPA, is significantly enhanced in the presence of fibrin, tPA may display fibrinolytic effects even in the presence of a high PAI level [25,26].
Furthermore, display of fibrinolytic activity can be explained by increased concentrations of uPA, which were demonstrated in the peritoneal fluid of our patients with peritonitis. The rise of uPA in the peritoneal fluid is probably not linked to mesothelium, but to endothelium, since uPA has not been demonstrated in mesothelial, but in endothelial cells of biopsies of inflamed appendices and in in vitro cultures, upon stimulation with inflammatory cytokines [27,28]. The more pronounced increase of uPA in peritoneal fluid compared with that in plasma, might be explained from the direction of secretion of uPA by endothelial cells, which is mainly abluminal [28]. Whether other uPA producing

or receptor bearing cells, like fibroblasts or macrophages, contributed to the fibrinolytic activity in the peritoneal cavity, remains to be clarified.
It is remarkable that plasma levels were not elevated in patients with peritonitis, except for a, relatively small, increase of uPA and FbDP. The findings of predominantly, normal plasma levels, as well as the absence of correlations between plasma and peritoneal fluid levels, suggest that the abdominal cavity with its surrounding peritoneum is a separate compartment. The coagulation and fibrinolytic responses to bacterial peritonitis in this compartment, depend on many factors, of which the individual contribution is difficult to assess. Intra-abdominal fibrinolysis occurs, as shown by increased FbDP levels. It seems, however, that the increase of fibrinolytic activity is limited by high PAI levels, and does not meet the overwhelming demand to overcome fibrin production. When the infectious stimulus is eliminated, fibrin formation probably stops. At that time residual fibrin has to be removed by means of the fibrinolytic system. For this, it is required that tPA and/or uPA levels in the peritoneal fluid remain elevated and PAI levels have decreased. Further studies will be conducted to assess the coagulation and fibrinolytic responses to elimination of the infectious focus.

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CHAPTER 9

FIBRINOLYTIC ACTIVITY IN THE ABDOMINAL CAVITY OF RATS WITH FAECAL PERITONITIS

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▲INTRODUCTION

Generalized peritonitis is a life-threatening condition that is poorly controlled by local defense mechanisms of the peritoneal cavity[1-4]. The outcome is usually fatal without surgical intervention if these protective systems fail. Removal of the source of the peritonitis, drainage of septic collections and extraction of fibrin deposits are essential to prevent residual abscesses. Fibrin deposits have a key role in abscess formation because bacteria entrapped in fibrin cannot be reached by phagocytes and antibiotics, and thus act as a nidus for infection[3,5-7].

Radical surgical debridement to achieve complete removal of fibrin was advocated by Hudspeth[8] but its benefits, namely reduction in the incidence of mortality and residual abscess, were not confirmed in a prospective randomized trial by Polk and Fry[9]. Scheduled relaparotomy has decreased the frequency of residual abscess, but this procedure is associated with a high risk of complications such as bleeding, bowel perforation and abdominal wall defects[10-12]. New fibrin deposits form immediately after each operation and it might be advantageous if they could be removed in a less traumatic way and subsequently prevented.

Deposition of peritoneal fibrin is a uniform host response to local inflammation and may be the result of increased fibrin formation and decreased fibrinolysis. Peritoneal fibrinolytic activity is reduced if there is direct injury to the peritoneum, ischaemia and bacterial peritonitis; mesothelial cell damage, leading to diminished release of tissue plasminogen activator (tPA), may be the main reason for decreased fibrinolysis[13-15]. Fibrinolytic activity might also be reduced by an increase in levels of plasminogen activator inhibitor (PAI), which has recently been demonstrated in inflamed peritoneum[16]. The activities of tPA and PAI in peritoneal fluid and the extent of peritoneal tissue damage were studied in rats with faecal peritonitis to determine their effects in fibrinolysis.

▲MATERIALS AND METHODS

Study design

Male Wistar rats weighing 250-280 gram were injected intraperitoneally with either 2 ml sterile normal saline (group 1, n=10) or 2 ml faecal suspension containing 10^4 colony forming units per ml of both *Escherichia coli (E. coli)* and *Bacteroides fragilis (B. fragilis)* (group 2, n=10). The rats were anaesthetized with a halothane-nitrous oxide-oxygen mixture 1 h after injection and via a midline laparotomy 0.4 ml peritoneal fluid was taken, snap frozen at -78°C in a polypropylene microfuge tube and stored at -80°C until assays of tPA and PAI activity were performed. A biopsy of the parietal peritoneum was taken for light microscopic examination. The abdominal cavity was debrided and rinsed thoroughly with saline solution. A relaparotomy was performed to take a peritoneal fluid sample and biopsy 3, 6, 24, 96 and 192 h after injection. Rats had free access to water and standard laboratory chow.

Bacterial faecal suspension

Fresh faeces of Wistar rats were collected, sterilized by steam (120°C, 20 min) and stored at 4°C before use.
E. coli strain ATCC 25922 was used for the experiment. Growth conditions and methods of preparation have been described previously[17]. *B. fragilis* from a clinical isolate stored at -70°C was prepared in the same way as *E. coli*, only Bacteroides bile esculin agar and Schaedler broth (Oxoid, Basingstoke, UK) were used.

Activities of tissue plasminogen activator and plasminogen activator inhibitor

Activities of tPA and PAI in the peritoneal fluid were measured by Coaset tPA assay (Kabi Diagnostica, Mölndal, Sweden) and Berichrom-PAI assay (Behring-werke, Marburg, Germany) respectively[18,19].

Histological examination

Sections of peritoneal biopsy specimens were stained with haematoxylin and eosin for light microscopical examination. On the basis of inflammatory infiltrate and the morphology of the mesothelial cells, peritoneal damage was scored semiquantitatively on a scale of 0-4 by one observer: 0, absent; 1, trace; 2, mild; 3, moderate; and 4, severe.

Statistical analysis

Results within a group were compared using analysis of variance (ANOVA) with the Friedmann test for non-parametric values. Data between the groups were analyzed using the Wilcoxon signed rank test. All tests were two-tailed; $p<0.05$ was considered significant.

▲RESULTS

All rats survived the experimental period. The mean weight loss was 5 (range 2-7) per cent and did not differ significantly among animals of either group. All rats that received a faecal suspension (group 2) had intra-abdominal fibrinous exudates after induction of peritonitis and intra-abdominal abscesses by day 8. These findings were not observed in animals of group 1.

Activity of tissue plasminogen activator

Median tPA activity in animals of group 1 was less than 1 unit/ml during the study. Activity of tPA in animals of group 2 remained less than 1.5 units/ml during the first 6 h of the experiment, reached a maximum 24 h after injection and subsequently decreased. Although activity in both groups was low, it tended to be higher in the rats of group 2 than in those of group 1. A significant difference was found at 24 h after injection ($p<0.05$) (Fig. 1).

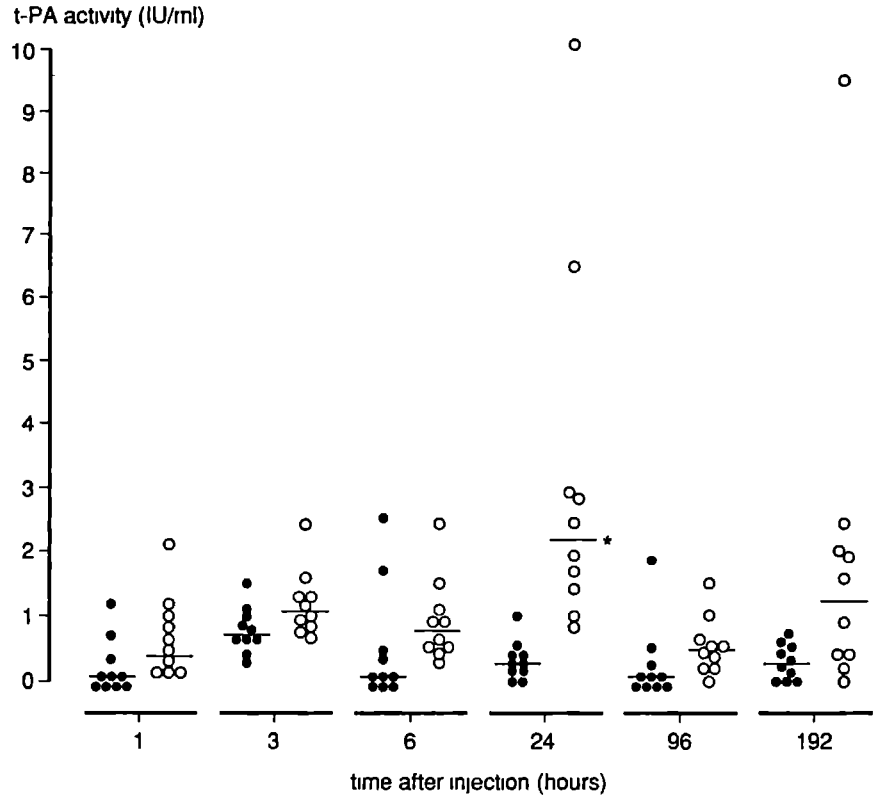

Figure 1 Activity of tissue plasminogen activator (tPA) in the peritoneal fluid of control rats (group 1, ●) and those given a faecal suspension (group 2, ○) Bars are median values * $p<0.05$ (group 2 *versus* group 1)

Activity of plasminogen activator inhibitor

Median PAI activity in animals of group 1 was less than 1 unit/ml at all determination points Activity of PAI in rats that received a faecal suspension (group 2) was increased within 3 h, reached a maximum between 6 and 24 h ($p<0.05$), and gradually declined during the following days Activity of PAI in rats of group 2 was significantly higher than in those of group 1 (Fig 2) ($p<0.001$)

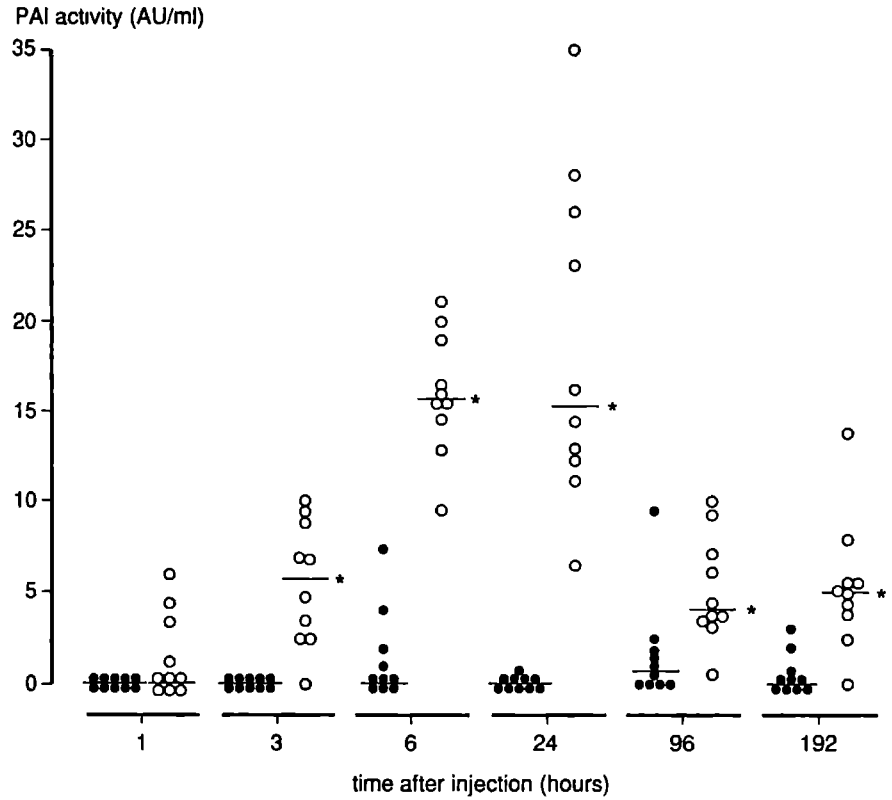

Figure 2 Activity of plasminogen activator inhibitor (PAI) in the peritoneal fluid of control rats (group 1,●) and those given a faecal suspension (group 2,○) Bars are median values * p< 0 001 (group 2 *versus* group 1)

Histological examination

No signs of peritoneal damage were identified in the biopsy specimens at 1 and 3 h after injection. There was no difference in the degree of peritoneal damage as assessed by median (range) score between the animals of both groups at 6 and 24 h after injection (group 2, 0.5(0-2) and 1(0-2) respectively; group 1, 1(0-2) at both times). Median (range) peritoneal damage scores at 96 and 192 h after

injection tended to be more severe in rats of group 2 (2(1-3) and 1(0-2) respectively) compared with those of animals in group 1 (0.5(0-2) and 0(0-2)). Activities of tPA and PAI in rats of group 2 differed significantly from those of animals in group 1 after stratification for histological score ($p<0.001$). Thus, tPA and PAI activity in the peritoneal fluid do not correlate with the severity of damage to the peritoneum.

▲DISCUSSION

The present study demonstrates that the main cause of change in abdominal fibrinolysis in rats with generalized peritonitis is a marked increase in the activity of PAI and that the enhanced activity of tPA is minor by comparison. Operative trauma alone did not significantly augment tPA or PAI activity.

Fibrinolysis in the abdominal cavity is dependent on the local activity of plasmin, which is determined by the balance between its generation from plasminogen and its inactivation by α_2-antiplasmin. The conversion of plasminogen into plasmin is stimulated by tPA, an enzyme that originates from various cells including those of mesothelial and endothelial type[20-22]. The activity of tPA is inhibited by PAI, which is also released by many cells throughout the body, including mesothelial cells[22].

In contrast to others[23-26], who measured fibrinolysis in the mesothelium, such activity was assessed in peritoneal fluid on the basis of the assumption that fibrin formation depends on the composition of this medium. It seems likely that, regarding intra-abdominal abscess formation, fibrinolytic activity of the abdominal exudate is a more appropriate parameter for overall fibrinolysis in the abdominal cavity than that of the mesothelium. Moreover, mesothelium cannot be isolated without being contaminated by the underlying fibrocollagenous tissue containing blood vessels, making meaningful measurements of fibrinolytic activity impossible.

The activity of tPA is dependent on the amounts of tPA and PAI antigen. Under normal circumstances tPA is released by mesothelial cells into the abdominal cavity[22,23,26]. The observed decline in fibrinolytic activity in inflamed peritoneum has been attributed to decreased release of tPA by the damaged mesothelium[15,25-28]. In contrast, decreased activity of tPA was not found in the peritoneal fluid of rats with generalized peritonitis. The increased activity of this enzyme that was

found even suggests enhanced release of tPA, although this supposition could not be confirmed because levels of tPA antigen could not be determined. Augmentation of tPA antigen levels seems likely in the rats with peritonitis because activity of tPA in these animals remained higher than in controls despite substantially increased PAI activity. These experimental data are concordant with clinical results in patients with peritonitis, in whom increased levels of tPA antigen in the peritoneal fluid were demonstrated[29].

The discrepancy between the present results and those of others who assessed fibrinolytic activity in peritoneal biopsy specimens may be explained by the prompt release of tPA from mesothelial cells into the peritoneal cavity after inflammation. This suggestion seems unlikely as Van Hinsberg et al.[22] reported decreased synthesis and release of tPA by mesothelial cells when inflammatory stimuli such as lipopolysaccharide and tumour necrosis factor (TNF) were added to mesothelial cell cultures. Therefore, tPA in the abdominal cavity may originate not only from the mesothelium but also from other tissues, such as the endothelium. This is corroborated by the findings of Suffredini et al.[30] and Paramo and colleagues[31], who found increased levels of tPA in the plasma of humans with endotoxaemia, sepsis and localized infection.

Inhibition of tPA by PAI seems to be the most important cause of decreased fibrinolysis in abdominal fluid. Vipond et al.[16] and Whawell and co-workers[32] reported reduced fibrinolytic activity in punch biopsies of inflamed peritoneum that contained high concentrations of PAI. The inhibitor in these biopsies may have been released by mesothelial cells challenged by lipopolysaccharide and/or TNF, as found in an *in vitro* study[22]. It may also have originated from connective tissue containing blood and blood vessels. This is supported by high plasma levels of PAI during peritonitis and sepsis[31,33]. These two sources became apparent in a recent study[34] in which production of PAI-1 was localized to mesothelium and serosal blood vessel endothelium in inflamed human appendix.

The activity of PAI was increased during the whole study period in rats with peritonitis, which was probably the result of ongoing infection as all animals had residual abscesses after 8 days. This finding suggests that fibrinolytic activity is impaired as long as infection is present.

The activities of tPA and PAI did not correlate with the histological findings. A similar degree of peritoneal damage during the first 24 h in animals of each group

was striking, whereas tPA and PAI activities in the peritoneal fluid showed significant differences. These observations support the likelihood of an extramesothelial source of tPA and PAI but do not exclude the mesothelium as responsible for production of peritoneal tPA or PAI. It has been demonstrated that mesothelial cells may release tPA and PAI without evidence of histological change[22].

The activities of tPA and PAI in the peritoneal fluid of rats with faecal peritonitis were increased; these changes were not the result of operative trauma. Markedly enhanced activity of PAI is probably the main cause of reduced fibrinolytic activity in the abdominal cavity.

Studies are warranted to assess the efficacy and safety of exogenous stimulation of peritoneal fluid fibrinolytic activity to overcome intrinsic inhibition by PAI and to reduce deposition of fibrin and formation of intra-abdominal abscesses.

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CHAPTER 10

IN VITRO STUDIES ON THE EFFECT OF HUMAN RECOMBINANT TISSUE TYPE PLASMINOGEN ACTIVATOR ON THE RAT FIBRINOLYTIC SYSTEM

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▲INTRODUCTION

Peritoneal fibrinolytic activity is depressed in peritonitis, which is held responsible for persistence of fibrin in the abdominal cavity and subsequent adhesion and abscess formation[1-3].
Tissue-type plasminogen activator (tPA) is an attractive agent to increase intra-abdominal fibrinolytic activity in order to prevent fibrin deposition. The high molecular weight and high affinity of tPA for fibrin tend to confine its activity to the abdominal cavity. Binding to fibrin also enhances catalytic efficacy of tPA and reduces its neutralization by plasminogen activator inhibitor (PAI)[4,5].
The rat is a suitable animal to study peritonitis and intra-abdominal abscess formation[6]. Rat tPA is preferred to modulate abscess formation in a rat model of peritonitis, but is not readily available. Human recombinant tPA (rtPA) seems to be a good alternative, since it is more readily available, its properties are established by clinical studies, and it has thrombolytic effects in animal models of thrombo-embolic stroke[7-9].
Prior to a study on the effects of intraperitoneally administered human rtPA on abscess formation in rats with peritonitis, we performed in vitro experiments to assess the effect of human rtPA on the rat fibrinolytic system.

▲MATERIALS AND METHODS

Human recombinant tPA (rtPA, Actilyse®, Boehringer Ingelheim, Alkmaar, The Netherlands) was obtained in vials of 50 mg rtPA had a specific activity of 580,000 IU/mg of protein. rtPA was dissolved in 5 ml sterile water and this stock solution was further diluted in Ringer's lactate solution (RLS) or methyl hydroxy propyl cellulose (MHPC) gel to obtain concentrations as used in the experiments.

MHPC gel was manufactured locally in vials of 2 ml sterile gel. MHPC gel was intended to be used as a vehicle of rtPA in the abdominal cavity, in order to achieve slow release of rtPA from the abdominal cavity, avoiding high systemic levels of rtPA.

Streptokinase (Streptase®, specific activity of 100,000 IU per 133-174 mg) was obtained

from Behringwerke, Marburg, Germany and dissolved in sterile water to prepare a stock solution of 3 mg/ml (range 2.7-3.5).

Human thrombin was provided by the Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands and dissolved in normal saline to prepare stock solutions of 50 and 80 IU/ml, respectively.

Batroxobin (Fibroclotin®) was purchased from Baxter, Hyland division, California, USA.

Normal pooled human plasma was prepared from plasma of 64 healthy hospital staff members, while normal pooled rat plasma was derived from plasma of 4 rats, as described previously[10].

Peritoneal fluid samples were taken from the abdominal cavity of rats and patients with peritonitis, immediately snap frozen, and stored at -80°C until assays were performed.

Four experiments were performed to determine the interaction between human rtPA and the rat fibrinolytic system.

(1) Capacity of human rtPA and streptokinase to lyse rat plasma clots, in comparison with human plasma clots.
(2) Effect of human rtPA on fibrinogen concentrations in rat plasma, as compared with human plasma.
(3) Effect of human rtPA on fibrin degradation in rat serum, in comparison with human serum, as measured by fibrin degradation products (FbDP).
(4) Inhibitory effect of rat PAI versus human PAI on human rtPA.

(1) Clot lysis capacity of human rtPA

Clot lysis capacity was measured in plasma in two ways. Firstly, by a method described previously which is based on the streptokinase resistance test of Moran et al.[10,11]. 20 μl of a series of plasminogen activator dilutions (rtPA or streptokinase) in 0.9% NaCl was added to 160 μl test plasma and the mixture was vortexed immediately. Then 20 μl of a thrombin solution (50 IU/ml) was added and the mixture was vortexed again. Clot formation occurred within 30 sec and lysis was established after exactly 10 minutes.

The plasma concentrations of rtPA or streptokinase that resulted in complete lysis of the clot within 10 minutes were recorded and expressed in μg/ml.
In the second method, a clot formed in the absence of plasminogen activator was overlaid by a solution of rtPA in either MHPC or RLS and the time required to achieve complete lysis of the clot was recorded.

(2) Effect of human rtPA on rat fibrinogen
Different concentrations (0 to 100 ng/ml) of rtPA were added to rat and human pooled citrated plasma, and after incubation for 15 minutes at 37°C a sample was taken to assess fibrinogen. Fibrinogen was measured by Clauss' method with human thrombin (80 IU/ml) as activator, using a KC10A coagulation analyzer (Amelung, Lemgo, Germany)[12].

(3) Effect of human rtPA on rat fibrin degradation
Different concentrations (0 to 100 ng/ml) of rtPA were added to rat and human pooled citrated plasma and incubated for 15 minutes at 37°C. Thereafter, plasma (200 μl) was recalcified (5.5 μl of 1 mol/L $CaCl_2$) and incubated with Batroxobin (15 μl stock solution) and thrombin (5 μl of 80 IU/ml) at 37°C for 15 minutes. After centrifugation at 14,000 g for 10 minutes the resultant sera were used to measure FbDP by Dade's latex immunoassay (Baxter, Miami, USA)[13].

(4) Inhibitory effect of rat PAI on human rtPA
Human rtPA was added to peritoneal fluid samples of two groups of rats, having a high (>15 AU/ml) and a low (<1 AU/ml) level of PAI, respectively. After incubation for five minutes at 37°C, rtPA activity was measured (added to a final concentration of 10 IU/ml) using Coaset-tPA assay (Kabi Diagnostica, Mölndal, Sweden). Two groups of patients, having a high and low level of PAI in their peritoneal fluid, respectively, served as controls.

▲RESULTS

Clot lysis capacity of human rtPA

Human rtPA was able to lyse rat plasma clots within 10 minutes at a minimum concentration of 0.75 μg/ml, compared to 0.5 μg/ml for human plasma clots (Table 1). Streptokinase did not lyse rat plasma clots at all up to concentrations of 300 μg/ml, while 50-150 μg/ml were sufficient to lyse the different human plasma clots. Clots made of a mixture of rat and human plasma lysed at a minimum concentration of 75 μg/ml, which excludes the possibility that streptokinase was neutralized by anti-streptokinase antibodies in rat plasma.

Table 1 Clot lysis by human recombinant tissue-type plasminogen activator (rtPA) and streptokinase (SK).
Three rat (RP) and two human plasma (HP) samples and one 1 1 mixture (RHP) sample were incubated with various (final) concentrations of rtPA or SK before clotting was induced by addition of thrombin Clot formation occurred within 30 sec and complete lysis (+) was established after exactly 10 min

	rtPA μg/ml						SK μg/ml					
	0 1	0 25	0 5	0 75	1 0	1 5	25	50	75	100	150	300
RP1	-	-	-	+	+	+	-	-	-	-	-	-
2	-	-	-	+	+	+	-	-	-	-	-	-
3	-	-	-	+	+	+	-	-	-	-	-	-
HP1	-	-	+	+	+	+	-	+	+	+	+	+
2	-	-	+	+	+	+	-	-	-	-	+	+
RHP	-	-	+	+	+	+	-	-	+	+	+	+

When plasma clots were overlaid with either rtPA/MHPC or rtPA/RLS mixtures at 0 5 mg/ml rtPA, no differences in clot lysis time were observed between the 2 diluents: all clots lysed within 150-165 minutes.

Effect of human rtPA on rat fibrinogen

Fibrinogen degradation was observed at ≥100 ng/ml of rtPA in rat plasma, while in human plasma this phenomenon already occurred at 33 ng/ml (Table 2).

Table 2 Degradation of plasma fibrinogen by rtPA
Different concentrations of rtPA (0, 0 3, 1, 3 3, 10, 33, and 100 ng/ml) were added to rat and human normal pooled plasma, and after incubation for 15 minutes at 37°C a sample was taken to assess fibrinogen

	Rat plasma	Human plasma
rtPA (ng/ml)	Fibrinogen (mg/ml)	Fibrinogen (mg/ml)
0	2.7	2.5
0 3	2 8	2 5
1	2 6	2 5
3 3	2 8	2 5
10	2 7	2 5
33	2 7	1 5
100	2 3	<1 0

Effect of human rtPA on rat fibrin degradation

Table 3 shows that FbDP could be demonstrated in the serum of rats in a rtPA dose-dependent manner. The measured FbDP concentrations were in general lower than those for the corresponding human samples.

Inhibitory effect of rat PAI on human rtPA

In peritoneal fluid samples with low PAI activity levels (<1 AU/ml) the recovery of rtPA activity was 66% for rat peritoneal fluid and 52% for human peritoneal fluid samples. In peritoneal fluid samples containing high levels of PAI activity (>15 AU/ml) the recovery was less than 5% (<0.5 IU/ml), both for rat and human samples.

Table 3 Degradation of fibrin by rtPA
Different concentrations of rtPA (0, 0 3, 1, 3 3, 10, 33, and 100 ng/ml) were added to rat and human normal pooled plasma and incubated for 15 minutes at 37°C Thereafter, clotting of the plasma was induced by adding Ca^{2+}, thrombin and batroxobin and clot lysis was allowed to continue for 10 minutes After centrifugation of the mixture, FbDP were measured in the supernatant

	Rat serum	Human serum
rtPA (ng/ml)	FbDP (μg/ml)	FbDP (μg/ml)
0	0	0
0 3	32	>1024
1	256	>1024
3 3	512	>1024
10	>1024	>1024
33	>1024	>1024
100	>1024	>1024

▲DISCUSSION

The fibrinolytic system of the rat is quite similar to that of humans, including components, such as plasminogen, α_2-antiplasmin, plasminogen activators, and plasminogen activator inhibitors[4 14 15]. This similarity also exists at the level of molecular function as indicated by cross-reactivity between rat factors and human rtPA observed in this study: (1) human rtPA activated rat plasminogen as deduced from clot lysis experiments and plasminaemic fibrinogen and fibrin degradation, and (2) human rtPA was inhibited by rat PAI as by human PAI. It seemed, however, that a rat clot is less susceptible to lysis by human rtPA in comparison with a human clot. In concordance, Korninger et al. found only 10% activity of human rtPA on rat plasminogen compared with human plasminogen[14]. The lower rat FbDP than human FbDP concentrations found in the present study might also be explained by the less efficient detection of rat FbDP in

A qualitative difference observed by us and others is the inability of streptokinase to lyse rat plasma clots, which rules out the possibility to use this agent in rat experiments[16]. It is concluded that human rtPA, dissolved in MHPC gel, is suitable to study intra-abdominal fibrinolytic therapy in the rat.

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CHAPTER 11

PHARMACOKINETICS OF HUMAN RECOMBINANT TISSUE TYPE PLASMINOGEN ACTIVATOR, ADMINISTERED INTRAABDOMINALLY, IN A RAT PERITONITIS MODEL

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▲INTRODUCTION

Peritonitis is associated with fibrin deposition in the abdominal cavity as part of the host response to inflammation[1]. Fibrin entraps bacteria, thereby preventing early bacteraemia. However, bacteria entrapped in fibrin cannot be reached by phagocytes and antibiotics, and thus fibrin acts as a nidus for intra-abdominal abscesses[2,3]. In patients with peritonitis, intra-abdominal abscess formation is a major cause of morbidity and mortality[4].

Fibrinolytic activators in the peritoneum are considered to play a key role in the resolution of fibrin deposits[5,6]. Fibrinolytic activity is reduced in peritonitis. This reduction has been attributed to a decrease in plasminogen activators and an increase in plasminogen activator inhibitors (PAI)[5-7]. Therefore, intra-abdominal administration of fibrinolytic agents, like human recombinant tissue-type plasminogen activator (rtPA), might be rational in an attempt to increase intra-abdominal fibrinolytic activity, and thus to prevent the development of abscesses.

There are only scarce data on the intra-abdominal use of rtPA in peritonitis[8]. The local fibrinolytic effect of rtPA may be influenced by PAI in the abdominal cavity during peritonitis. A potentially adverse systemic effect of rtPA, as bleeding, may be abolished by PAI in the plasma during peritonitis.

In order to obtain information on the fibrinolytic activity of rtPA in peritonitis, we first measured endogenous tPA and PAI activity in the peritoneal fluid and plasma of rats with peritonitis. Subsequently, we investigated the pharmacokinetics of rtPA, administered intra-abdominally, in such rats.

▲MATERIALS AND METHODS

Materials

Human recombinant tPA (rtPA, Actilyse®) was kindly donated by Boehringer Ingelheim, Alkmaar, The Netherlands, in vials of 50 mg. This rtPA had a specific activity of 580,000 IU/mg of protein. rtPA was dissolved in 5 ml sterile water and this stock solution was diluted in sterile methyl hydroxy propyl cellulose (MHPC) gel to obtain concentrations

of 0.5 and 2 mg/ml rtPA, respectively. Sterile MHPC gel was manufactured locally. MHPC was used with the intention to obtain slow release of rtPA. In vitro, MHPC gel did not interfere with rtPA activity. The rtPA/MHPC mixtures were vigorously vortexed before use.

Design of the study

This study consisted of two experiments. In experiment 1, the endogenous tPA activity and PAI activity were measured in the peritoneal fluid and plasma of rats with peritonitis, and compared with a control group of rats without peritonitis. In experiment 2, tPA antigen and activity in these compartments were assessed after intraperitoneal administration of a single dose of rtPA, in rats with and without peritonitis.

Experiment 1

Male Wistar rats, weighing 250-280 gram, were injected intraperitoneally with either 2 ml sterile saline solution (n=8; group 1) or 2 ml sterile faecal suspension mixed with 10^4 colony forming units/ml of both *Escherichia coli* and *Bacteroides fragilis* (n=13; group 2). After one hour, the rats were anaesthetized and a midline laparotomy was performed to debride and lavage the abdominal cavity. After abdominal closure, 5 ml of normal saline were administered subcutaneously for resuscitation. This experimental model has previously been described in more detail[9].

At 1, 2, 4, 8, 16, 32 hours (group 1), and at 1, 2, 4, 8, 16, 32, 64, 128 and 192 hours (group 2) after injection, rats were anaesthetized and a peritoneal fluid sample was taken for assessment of tPA and PAI activity. Due to small amounts, no peritoneal fluid was recovered at 64, 128 and 192 hours after injection in rats of group 1. In both groups, blood samples were taken before and at 1, 2, 4, 8, 16, 32, 64, 128 and 192 hours after injection. 5 ml of saline were administered subcutaneously to compensate for fluid losses due to blood sampling.

Experiment 2

The same model as described above was used in 20 rats; 10 were injected with saline (control group) and another 10 with the faecal suspension (peritonitis group). During laparotomy, half of the rats in each group received 2.5 ml of MHPC gel containing 0.5 mg/ml rtPA, the other half 2.5 ml of MHPC gel containing 2.0 mg/ml rtPA. At 1.5,

2, 4, 6, 8 and 24 hours after injection, a peritoneal fluid and a blood sample were taken to measure tPA antigen and tPA activity. Since the peritoneal fluid recovered from rats in the control group was not sufficient for both measurements, only tPA antigen was measured in this group.

In addition, the influence of MHPC gel on plasma tPA antigen levels was assessed. To investigate this, another five rats with faecal peritonitis received 0.5 mg/ml rtPA dissolved in Ringer's lactate solution (RLS) and their plasma tPA antigen levels were compared with those of rats in the peritonitis group, given 0.5 mg/ml rtPA in MHPC gel.

During the experiments rats had free access to water and standard rat chow (MH-B 1010, Hope Farms, Woerden, The Netherlands). Experiments were performed following guidelines of the animal experiments ethical committee.

Samples

Peritoneal fluid samples (0.2-0.5 ml) were snap frozen at -80°C immediately after percutaneous aspiration from the abdominal cavity. All samples were thawed for exactly 5 minutes at a temperature of 4°C prior to use.
Blood samples (0.45 ml), drawn by cardiac puncture, were collected into 0.1 volume of ice-cold 0.109 M citrate, pH 6.0, cooled for 5 minutes and centrifuged at 14,000 g (Eppendorf centrifuge 5415-c) for 5 minutes to prepare platelet free plasma. The plasma samples were stored at -80°C.

Assays

tPA antigen was measured by ELISA (Asserachrom-tPA, Boehringer, Mannheim, Germany). The assay measures both complexed (to PAI) and uncomplexed tPA. Rat tPA antigen cannot be measured by this assay.

tPA activity was measured on euglobulin fractions using the Coaset-tPA assay (Kabi Diagnostica, Mölndal, Sweden) described by Nilsson et al.[10,11]. The assay was performed

on euglobulin fractions which were made by acidification of plasma samples or peritoneal fluid samples mixed with an equal volume of tPA-poor plasma. The acidification procedure was rigorously standardized and was performed by adding 15 μl of 0.044 N acetic acid to 200 μl of sample on ice, followed by incubation for 30 minutes and centrifugation at 14,000 g for 10 minutes to obtain the precipitate. The euglobulin fractions were incubated in a suitable dilution (usually 1:100) with plasminogen in the presence of a tPA stimulator at 37°C for 3 hours. Plasminogen activation was monitored by the presence of S2251, a chromogenic substrate specific for plasmin, by reading the increase in absorbance at 405 nm. The assay measured both human and rat tPA activity and was calibrated with a tPA standard which is expressed in International Units (IU), assessed by calibration against the International Standard for tPA (NIBSC standard). The assay procedure resulted in full recovery of rtPA, spiked into pooled normal human plasma for 15 minutes (range 94-110%).

PAI activity was measured by Berichrom-PAI assay (Behringwerke, Marburg, Germany) in peritoneal fluid and plasma samples mixed with one or more volume(s) of PAI-deficient plasma. In this assay the sample is incubated with a fixed amount of urokinase at 37°C during 5 minutes, after which remaining urokinase activity is measured via plasminogen activation and subsequent hydrolysis of a chromogenic substrate[12]. PAI activity is expressed in Arbitrary Units (AU) where 1 AU/ml sample is able to inhibit 1 IU/ml of urokinase in 5 minutes.

Statistical analysis

Median values and 95% confidence intervals (CI) or ranges are given. Results were analyzed using the Friedmann test with Duncan's correction for multiple comparison within groups and using the Mann Whitney-U test for comparison between groups after calculation of the area under the curve (AUC). A p-value less than 0.05 was considered significant.

▲RESULTS

Experiment 1

In rats of group 1, levels of tPA activity in the peritoneal fluid and plasma were less than 2 IU/ml and 6 IU/ml, respectively, at all determination points.

In rats of group 2, tPA activity in the peritoneal fluid only slightly but significantly increased up to 64 hours after inoculation and subsequently declined (Figure 1).

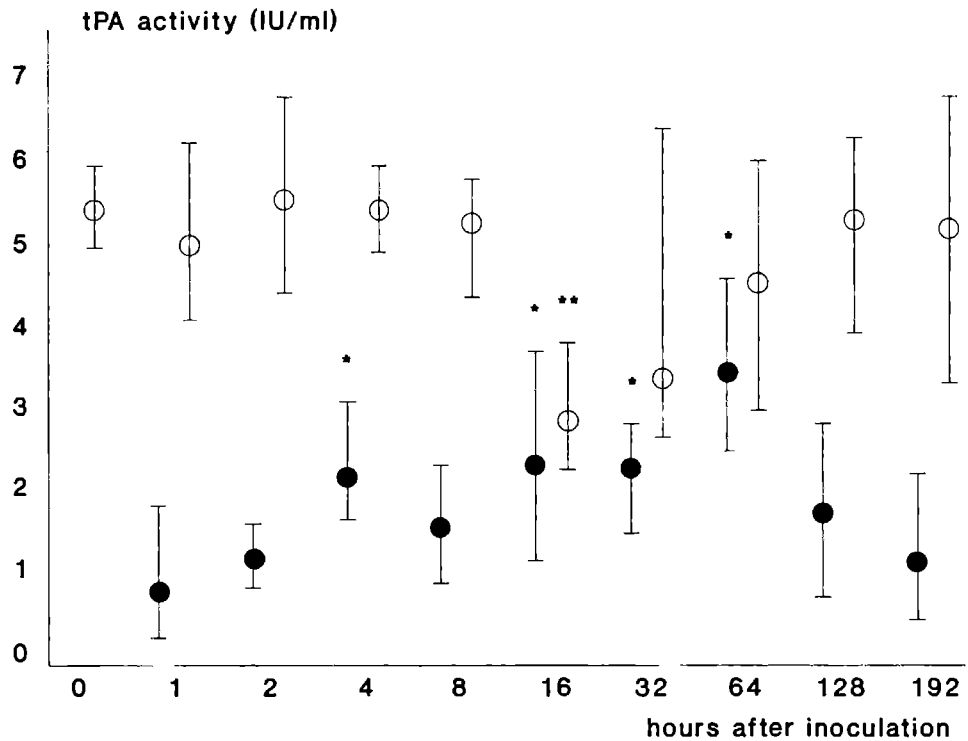

Figure 1. Activity of tissue-type plasminogen activator (tPA) in the peritoneal fluid (●) and plasma (○) of rats inoculated with a faecal suspension. Median values and 95% confidence intervals are given. * $p<0.05$; 4, 16, 32 and 64 hours *versus* 1 hour after inoculation. ** $p<0.05$; 16 hours *versus* before (0) and 1, 2, 4 and 8 hours after inoculation.

The AUC of tPA activity in the peritoneal fluid of animals of group 2 was significantly higher than in animals of group 1, between 1 and 32 hours after inoculation. Median plasma tPA activity remained about 5.5 IU/ml during the first 8 hours of the experiment, dropped to a minimum at 16 hours after injection of the faecal suspension and subsequently recovered (Figure 1). There was no significant difference in plasma tPA activity between both groups.

In rats of group 1, levels of PAI activity in the peritoneal fluid and plasma remained less than 4 AU/ml and 2 AU/ml, respectively, during the whole experimental period. PAI activity in the peritoneal fluid of rats of group 2 steadily increased, reached a maximum at 16 hours after inoculation and subsequently decreased (Figure 2).

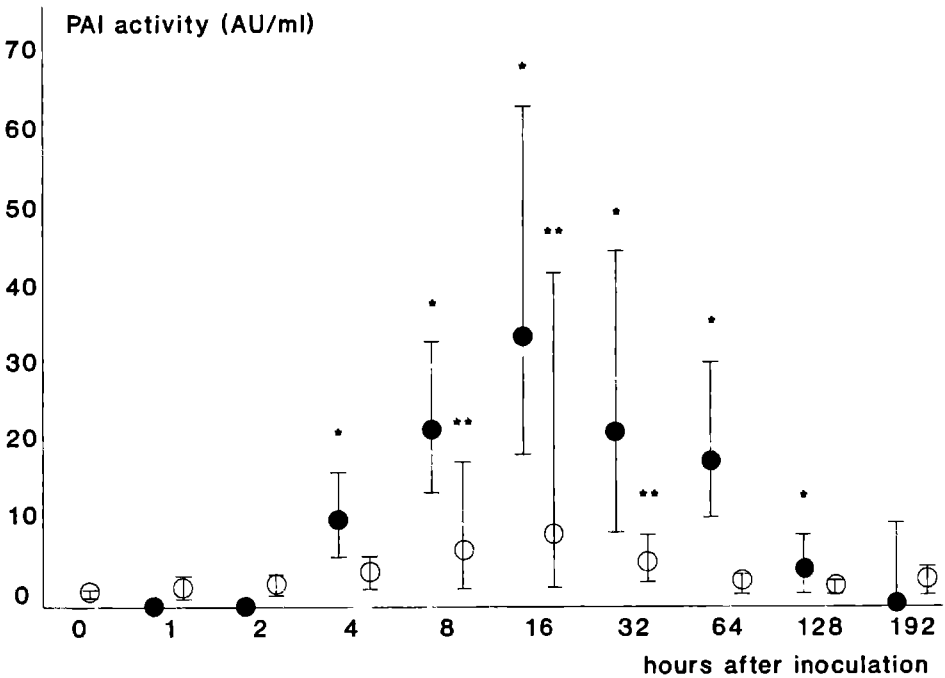

Figure 2. Activity of plasminogen activator inhibitor (PAI) in the peritoneal fluid (●) and plasma (○) of rats given a faecal suspension. Median values and 95% confidence intervals are given. * $p<0.05$; 4, 8, 16, 32, 64 and 128 hours *versus* 1 hour after injection. ** $p<0.05$; 8, 16 and 32 hours *versus* before (0) and 1 hour after injection.

The AUC of PAI activity in group 2 was significantly higher than that in group 1, between 1 and 32 hours after inoculation.
Plasma PAI activity in rats of group 2 showed a similar pattern as that in the peritoneal fluid (Figure 2). The AUC of plasma PAI activity in these rats was significantly higher than in rats of group 1.

Experiment 2

tPA antigen levels in the peritoneal fluid of rats in the peritonitis group and in the control group were approximately the same. These levels showed a similar pattern of continuous decrease in time starting with levels above 100,000 ng/ml for both doses used. The biological half life of rtPA dissolved in MHPC gel was approximately two hours.
tPA activity in the peritoneal fluid of rats with peritonitis was dose-dependent and continuously decreased in time starting with median levels of 60,000 and above 100,000 IU/ml at the dose of 0.5 mg/ml and 2 mg/ml rtPA, respectively.
The measured tPA antigen levels, the tPA activity calculated from tPA antigen levels and the specific activity of rtPA (580,000 IU/mg), and the measured tPA activity, at the dose of 0.5 mg/ml rtPA, are shown in Figure 3. The measured tPA activity tended to be lower than the calculated tPA activity, reaching a significant difference ($p<0.05$) at 8 and 24 hours after inoculation. A similar, but less pronounced difference, was observed at the dose of 2.0 mg/ml rtPA (data not shown). As mentioned before, tPA activity was not measured in the peritoneal fluid of control rats due to insufficient amounts of fluid.
Plasma tPA antigen levels showed a dose-dependent increase after administration of 0.5 and 2.0 mg/ml rtPA in MHPC gel followed by a decline in both rats with peritonitis and control rats (Table 1). Maximum levels of tPA antigen at both doses of rtPA tended to be lower and showed to occur earlier in rats with peritonitis than in control rats. In both groups of animals, plasma tPA activity levels remained below 6 IU/ml during the first 8 hours and even decreased to below 1 IU/ml at 24 hours, and these showed no dose-level relationship. Similar to peritoneal fluid, the measured tPA activity in the plasma of rats in the peritonitis group was markedly lower than the calculated tPA activity. Surprisingly, this relative deficit in tPA activity was also found in the plasma of the control group.

Median plasma levels of tPA antigen at 1.5, 2, 4, 6, 8 and 24 hours after injection in rats with peritonitis, given 0.5 mg/ml rtPA in RLS, are listed in Table 1. The AUC of tPA antigen in rats given rtPA in RLS was significantly higher than that in rats given rtPA in MHPC gel.

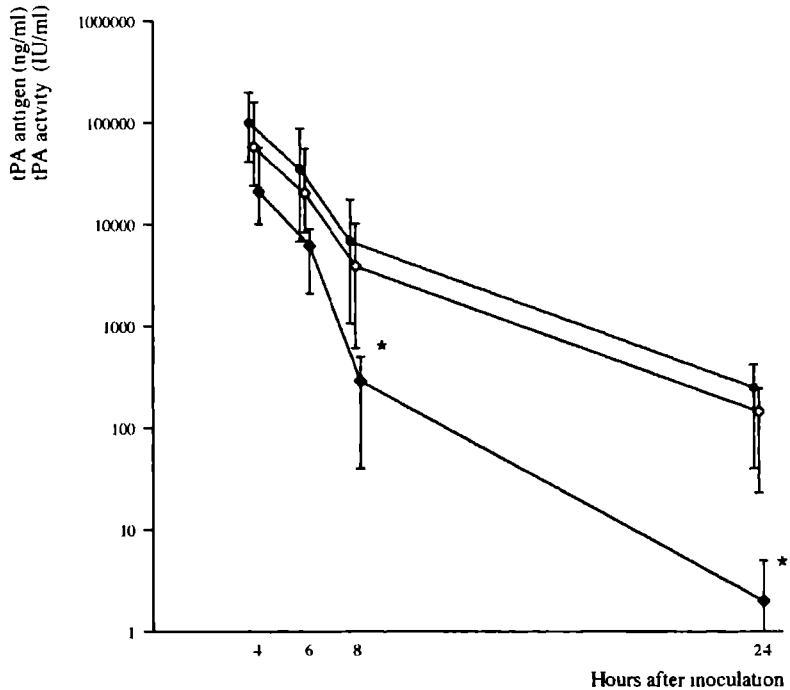

Figure 3 Median values and ranges of tPA antigen (•), calculated tPA activity (◇), and measured tPA activity (◆) in the peritoneal fluid, after administration (one hour after inoculation) of 0 5 mg/ml rtPA, dissolved in MHPC gel, in rats of the peritonitis group
* p<0 05, calculated *versus* measured tPA activity at 8 and 24 hours after inoculation.

▲DISCUSSION

This study demonstrates that the fibrinolytic activity in the abdominal fluid of rats with faecal peritonitis can be raised substantially up to 24 hours after a single dose of human

Table 1. Plasma levels of tPA antigen (AG) at the doses of 0.5 and 2 mg/ml rtPA dissolved in MHPC gel, and at the dose of 0.5 mg/ml rtPA dissolved in RLS administered intraperitoneally one hour after inoculation (a.i.), in rats with peritonitis and control rats (5 rats in each group). Median values and ranges are given.

	dose mg/ml	1 5 hours a i.	2 hours a i	4 hours a i	6 hours a i	8 hours a i	24 hours a i
tPA Ag plasma ng/ml	0 5	13	27	32	25	15	3
peritonitis rats	MHPC gel	(8-16)	(15-32)	(27-39)	(20-42)	(8-20)	(0-5)
	2	50	90	52	130	77	14
	MHPC gel	(16-100)	(20-480)	(20-106)	(70-520)	(21-240)	(5-20)
	0 5	6	20	56	315	213	10
	RLS	(4-9)	(15-30)	(42-80)	(273-368)	(145-250)	(5-21)
tPA Ag plasma ng/ml	0 5	3	4	8	27	76	3
control rats	MHPC gel	(0-5)	(4-25)	(3-30)	(16-36)	(23-84)	(2-5)
	2	24	30	190	470	1100	50
	MHPC gel	(17-33)	(21-42)	(62-240)	(170-520)	(470-2000)	(20-176)

rtPA, administered intraperitoneally, despite simultaneously high levels of inhibitor of fibrinolytic activity.

Rotstein et al. demonstrated that intra-abdominal administration of 0.01 mg/ml human rtPA in Hanks balanced salt solution prevented abscess formation in an infected human fibrin clot model[8]. In the same model high dose rtPA (0.1 mg/ml) prevented abscess formation following a 6-hour delay in administration[13]. They did not report on intra-peritoneal tPA activity or PAI activity.

Previously, we have performed a pilot experiment in normal rats, given 0.1 mg/ml human rtPA dissolved in MHPC gel intraperitoneally. In this experiment, tPA antigen concentrations in the peritoneal fluid were 5000 ng/ml, 10 ng/ml and 0 ng/ml at 0.5, 8 and 24 hours, respectively, after administration of rtPA. Corresponding plasma levels were zero.

In the present study higher doses than 0.1 mg/ml rtPA were tested for three reasons. First, rat fibrin is less susceptible to human rtPA than human fibrin, which was used in the fibrin clot model of Rotstein and co-workers[14]. Second, higher amounts of intra-abdominal fibrin were expected in the present model, as compared with the fibrin clot

model, due to the difference in severity of peritonitis. Third, the dose of 0.1 mg/ml rtPA resulted in very low tPA antigen levels in the abdominal cavity, particularly at 8 and 24 hours. These levels were not expected to be sufficient to lyse rat fibrin.
High tPA antigen levels were obtained in the abdominal cavity in the present experiment, especially during the first six to eight hours after administration of rtPA. However, measured tPA activity levels in peritonitis were much lower than inferred from antigen levels and the specific activity of the rtPA agent (580,000 IU/mg pure protein). Inhibition by PAI might explain the differences between the calculated and measured tPA activity[15]. This is supported by the simultaneity in the most pronounced differences in calculated and measured tPA activity levels in the peritoneal fluid of rats with peritonitis in the second experiment, and the highest endogenous PAI activity levels in the peritoneal fluid of those in the first experiment. Moreover, the differences between the calculated and measured tPA activity levels tended to be smaller at the high dose (2.0 mg/ml) rtPA.
Plasma levels of tPA antigen in rats with peritonitis tended to be lower than those in control rats. First, this might be due to the high affinity of rtPA for the intra-abdominal fibrin deposits of rats with peritonitis[16]. Second, rtPA that is complexed to intra-abdominal PAI, might not enter the circulation in these rats. The use of MHPC gel has contributed to low plasma levels of tPA antigen in rats with peritonitis.
The relatively low levels of tPA antigen and particularly of tPA activity in the plasma may be a favourable aspect of the intra-abdominal use of rtPA in peritonitis, reducing the risk of bleeding, a potential side effect of rtPA. However, these findings do not reassure that bleeding cannot occur, as these may occur locally within the peritoneal cavity following surgery and sepsis. In another experiment in rats with faecal peritonitis, we found normal plasma levels of fibrinogen at the dose of 2.0 mg/ml rtPA, and even increased fibrinogen levels at the dose of 0.5 mg/ml rtPA.
Analogous to peritoneal fluid, the observed increased plasma levels of endogenous PAI might be responsible for the low levels of tPA activity found in plasma. However, forming an inactive complex with PAI does not explain the low plasma tPA activity in control rats, since plasma PAI activity in these rats was low. Other circulating inhibitors of tPA than PAI, as described in mice, might explain this discrepancy between tPA antigen and activity levels[17].
In comparison with 0.5 mg/ml rtPA, the dose of 2 mg/ml rtPA resulted in higher levels of tPA activity in the abdominal cavity but not in plasma. Based on these results,

2 mg/ml rtPA might be preferred for further experiments.
Finally, we would like to take into consideration that in this study a mildly acidic citrate anticoagulant (pH 6 0) in stead of one of the newly recommended strongly acidic citrate anticoagulants (pH 4.0-4.4) was used for blood sampling to prevent ex vivo complex formation between PAI and tPA or urokinase-type plasminogen activator[18 19]. Although in normal citrated plasma the tPA recovery was about 100% (see tPA activity assay), a recent study in patients with peritonitis showed that the more acidic citrate anticoagulant (Stabilyte® tubes) resulted in a 2.3-fold (range 1.4- to 6.0-fold) higher level of tPA activity[20]. Thus, plasma levels of tPA activity might have been underestimated in the present study. For peritoneal fluid samples we did not use an anticoagulant at all, but we snap froze these samples immediately after aspiration and used minimal thawing times before measurements. Theoretically this procedure also might have resulted in underestimation of tPA activity levels in the peritoneal fluid. Comparison of different sampling procedures in a recent study on patients with peritonitis suggests that peritoneal fluid levels are not affected by the anticoagulant method used, possibly because of the acidic nature of the peritoneal fluid in peritonitis[21].

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CHAPTER 12

EFFECT OF RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR ON INTRAABDOMINAL ABSCESS FORMATION IN RATS WITH GENERALIZED PERITONITIS

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▲INTRODUCTION

Fibrin deposition in the abdominal cavity is an integral host reaction to intra-abdominal inflammatory processes. In one respect, it has beneficial effects, walling off infectious foci thus localizing infection and preventing early systemic spread of bacteria[1-4]. However, it has deleterious effects on the intra-abdominal defense mechanisms. Fibrin deposits prevent the clearance of bacteria from the abdominal cavity by occluding the stomata in the diaphragm. Moreover, bacteria that are caught in fibrin are not accessible to the immunologic defense mechanisms and to antimicrobial agents[5-7]. The colonized fibrin clots are a nidus for abscess formation, which is an important cause of morbidity and mortality in patients with generalized peritonitis[8,9]. Therefore, a thorough debridement of the abdominal cavity that removes all fibrin deposits, is of utmost importance in the operative treatment of patients with generalized peritonitis. However, surgical debridement is often incomplete and has a high risk of complications, such as bleeding and intestinal perforation[10]. Moreover, new fibrin deposits will be formed immediately after operation.

Fibrin deposition depends on the balance between fibrin formation and fibrinolysis. During peritonitis, intra-abdominal fibrin formation is enhanced, whereas fibrinolytic activity is reduced[11-13]. Therefore, exogenous stimulation of intra-abdominal fibrinolysis could be of benefit in preventing abscess formation.

The present study was designed to determine first, if topically applied recombinant tissue plasminogen activator (rtPA), which is a potent stimulator of fibrinolysis[14], is able to prevent intra-abdominal abscess formation in rats with generalized peritonitis, and second, if intra-abdominal use of rtPA is safe with respect to complications, such as sepsis and bleeding.

▲MATERIALS AND METHODS

Design of the study

The study consisted of two consecutive experiments. In experiment 1, the effects of rtPA on intra-abdominal abscess formation and bacteraemia were assessed. In

experiment 2, the effect of rtPA on early bacteraemia was studied, using a higher dose of bacteria in the inoculum as compared with experiment 1.

Experiment 1

Male Wistar rats, weighing 250 to 280 g, were injected intraperitoneally with 2 ml of a faecal suspension containing 10^4 colony forming units (cfu) per ml of *Escherichia coli* (*E. coli*) and 10^4 cfu per ml *Bacteroides fragilis* (*B. fragilis*). One hour after inoculation, the rats were anaesthetized with a halothane-nitrous oxide-oxygen mixture. Through a midline laparotomy, the abdomen was debrided, which included a partial omentectomy, and was thoroughly rinsed with normal saline solution. Before closure of the abdomen, the rats were randomly assigned to one of three treatment groups. Group 1 (n= 20) received 2.5 ml Ringer's lactate solution intraperitoneally, group 2 (n= 20) received 2.5 ml methyl hydroxy propyl cellulose (MHPC) gel, and group 3 (n= 32) received 2.5 ml MHPC gel containing 0.5 mg per ml rtPA (kindly provided by Boehringer Ingelheim, Alkmaar, The Netherlands). MHPC gel was used as a vehicle. The abdomen was closed in two layers with running 4x0 polypropylene sutures. Postoperatively, 5 ml of normal saline solution were administered subcutaneously for resuscitation. Following operation, the rats had free access to water and standard rat chow (MH-B 1010, Hope Farms, Woerden, The Netherlands). At zero, six, 12 and 24 hours after laparotomy, blood samples were taken under general anaesthesia to assess bacteraemia. The rats were weighed at day zero and day 5. At day 5, the surviving rats were sacrificed by intracardiac pentobarbital injection. In all rats, the abdomen was reopened using sterile conditions and meticulously inspected for abscess formation and haematomas. All intra-abdominal collections containing "purulent" material were counted as abscesses. The abscess localization was noted and, if present, abscesses at different spots were excised for culture.

Experiment 2

The aforementioned model of intra-abdominal infection was used, but 10^8 cfu per ml *E. coli* was instilled instead of 10^4 cfu per ml *E. coli*. At laparotomy, the rats received either 2.5 ml of MHPC gel (n=8; control group) or 2.5 ml of MHPC gel containing 0.5 ml per mg rtPA (n=11; rtPA group). At zero, one, three, six and 12 hours after laparotomy, blood samples were taken under general anaesthesia for culture. At the same time, 5 ml of sterile saline solution were injected subcutaneously for resuscitation.

Bacterial faecal suspension

E. coli strain American Type Culture Collection (ATCC) 25922 and *B. fragilis* from a clinical isolate were used in a concentration of 10^4 cfu per ml Schaedler broth (Oxoid, Hampshire, United Kingdom) in experiment 1. In experiment 2, *E. coli* was used in a concentration of 10^8 cfu per ml. Bacteria were cultured according to standard methods described previously[13].
Fresh faeces of Wistar rats were weighed and 1:2 suspended into pyrogen free water. After homogenization, the suspension was sterilized by steam (121 degrees C for 20 minutes) and centrifuged at 2,000 revolutions per minute for 15 minutes. The supernatant was homogenized, resterilized and stored at 4 degrees C. Immediately before intraperitoneal injection, the faecal suspension was mixed with the bacterial suspensions.

Bacteria within abscesses

The excised abscesses were minced in Brain Heart Infusion (BHI) broth (Oxoid, Hampshire, United Kingdom) and in Schaedler broth for aerobe and anaerobe culturing, respectively, and were subsequently outplated. After 24 (aerobes) and 48 (anaerobes) hours of incubation at 37 degrees C, any growth was identified according to standard procedures.

Blood cultures

Blood samples (0.45 ml) were taken by cardiac puncture under general anaesthesia and collected in sterile tubes containing 0.05 ml buffered citrate. Serial dilutions in BHI broth were made and plated onto both aerobic (Mac Conkey, blood agar; Oxoid, Hampshire, United Kingdom) and anaerobic (Bacteroides bile esculin agar, Brucella blood agar; Becton-Dickinson, Baltimore, USA) plates. After 24 and 48 hours of incubation at 37 degrees C, bacteria were counted and identified. Bacterial concentration was expressed in ^{10}log cfu per ml whole blood.

Statistics

Statistical analysis was performed using chi-square test, and Student's *t* test or Kruskall Wallis rank analysis, when appropriate. When p-values were less than 0.05, a difference between groups was considered significant.

▲RESULTS

Experiment 1

All rats lost weight during the experimental period. Average (± SD) weight loss at day 5 was 8.0 percent (± 2.9) in group 1, 7.3 percent (± 2.2) in group 2, and 8.5 percent (± 1.8) in group 3. The differences between groups were not statistically significant. The mortality rate in group 3 (ten of 32 rats, 31 percent) was significantly higher ($p<0.01$) than in groups 1 and 2 (two of 40, 5 percent). None of the ten rats in group 3 that died had intra-abdominal abscesses at autopsy. All of these rats died between 12 and 24 hours after laparotomy. Rats in group 1 and 2 died three days after laparotomy and had multiple intra-abdominal abscesses at autopsy. No rat died due as a result of intra-abdominal bleeding.

All rats in group 1 (20 of 20 rats) and group 2 (20 of 20 rats) had intra-abdominal abscess, in contrast to only one-half of those in group 3 (16 of 32 rats). Of the 22 surviving rats in group 3 that had received rtPA, six (27 percent) did not have any abscesses. The number of abscesses in the 16 rats in group 3 that bore abscesses was significantly lower when compared with group 1 or group 2 (Table 1). The localization of abscesses differed also between rats in group 3 and rats in groups 1 and 2. Whereas abscesses in 15 of 16 rats of group 3 were found in the subdiaphragmatic space and around the liver, 34 of 38 rats in groups 1 and 2 showed abscesses spread over the whole abdominal cavity.

The bacterial species cultured from the intra-abdominal abscesses are listed in Table 2. *E. coli* (ATCC 25922) was found in most of the abscesses. In contrast, *B. fragilis* was only cultured from abscesses in one rat. Three rats had sterile abscesses, two in group 1, and one in group 3. A *Proteus mirabilis* species was cultured in 32 percent of the rats in group 1, in 37 percent of group 2, and in 25 percent of group 3. Other than *E. coli* (ATCC 25922) and *B. fragilis*, all species isolated from the abscesses

were present in the faecal contents obtained from the caecum of these rats (data not shown)
All rats had negative blood cultures at all determination points None of the rats had intra-abdominal haematomas

Table 1 Effect of rtPA on abscess formation in surviving rats

group	no of rats with abscesses (total no)	no of abscesses per rat median (range)
1	19 (19)	10 (2-25)
2	19 (19)	12 (4-20)
3	16 (22)	3 (1-8)*

* p<0 01, group 3 *vs* group 1 and 2

Experiment 2
In none of the blood samples, which were taken immediately before and one hour after laparotomy, bacterial growth was detected Three hours after laparotomy, only one rat in the rtPA group had a positive blood culture Six hours after laparotomy, eight (73 percent) of 11 rats in the rtPA group had a positive blood culture, compared with none (zero percent) of the eight rats in the control group The median (range) blood bacterial concentration in the rtPA group was 10^3 (zero to 10^5) Twelve hours after laparotomy, all rats in both groups had a positive blood culture The median (range) blood bacterial concentration in the rtPA group was 10^6 (10^4 to 10^8) and in the control group, 10^5 (10^3 to 10^7) In all rats with positive blood cultures, only *E coli* (ATCC 25922) was isolated from the blood None of the rats in either group survived for more than 24 hours after laparotomy

▲DISCUSSION

The present study demonstrates that enhancement of the intra-abdominal fibrinolytic

Table 2 Bacteriology of intra-abdominal abscesses in surviving rats

group	no of rats	no (%) of rats with positive cultures			
	with abscesses	*E coli*	*B fragilis*	*P. mirabilis*	other*
1	19	17 (89)	0 (0)	6 (32)	3 (16)
2	19	19 (100)	0 (0)	7 (37)	4 (21)
3	16	13 (81)	1 (6)	4 (25)	2 (13)

E coli, *Escherichia coli*; *B fragilis*, *Bacteroides fragilis*, *P mirabilis*, *Proteus mirabilis*
*other species are *Enterococci*, *Staphylococci epidermidis*, *E coli* (other than ATCC 25922) and anaerobe gram-positive rods

activity by intra-abdominal administration of rtPA substantially reduces the number of abscesses in rats with generalized peritonitis. Entrapment of bacteria in fibrin in the abdominal cavity is a significant cause of abscess formation in generalized peritonitis[2]. As operative treatment alone cannot prevent intra-abdominal abscess formation, enhancement of intra-abdominal fibrinolysis by rtPA to optimize removal of fibrin might be a beneficial adjunct to operative treatment. This supposition is based on recently published studies of Rotstein and others, who showed that rtPA prevents abscess formation in rats in whom colonized fibrin clots were implanted intra-abdominally[15,16]. Because their model, using human fibrin clots in rats, has limited similarity to the clinical situation, we used a model of generalized peritonitis, in which residual intra-abdominal abscesses occur in nearly 100 percent of rats. In our opinion, this approach has more clinical relevance.

In the experiments, we used a dose of 0.5 mg per ml rtPA. This dose was based on the results of previously performed studies by our group[17]. This dose resulted in high intra-abdominal levels of tPA activity during eight hours after instillation and only slightly increased plasma tPA activity levels. Plasma tPA activity levels probably were low because of a slow release of rtPA from the abdominal cavity due to its high molecular weight and its high affinity for fibrin[18]. Furthermore, tPA might be inhibited by high plasma levels of plasminogen activator inhibitor. These were demonstrated by Paramo

and co-workers in patients with bacterial infections[19]. Methyl hydroxy propyl cellulose gel was used as a vehicle to further reduce the release of rtPA from the abdominal cavity[17]. Moreover, it has been suggested that cellulose gel prevents adhesion formation. This effect of cellulose gel has been attributed to mechanical separation of the bowels and high local activity of plasminogen activator on peritoneal surfaces[20,21]. However, we were not able to demonstrate a favourable effect of MHPC gel alone on abscess formation, whereas gel containing rtPA reduced the abscess rate, particularly for abscesses localized between the bowels.

Cultures from the abscesses revealed two interesting observations. First, *B. fragilis* was not cultured from the abscesses. The absence of *B. fragilis* in the abscesses might be explained by the fact that the abdominal concentration of this anaerobe had been reduced substantially after operative treatment[22]. Secondly, the presence of *P. mirabilis* and other species in the abscesses, originating from the intestines of the rats, showed the occurrence of bacterial translocation. A similar observation has been reported previously by other authors in experimental and clinical studies[23-26].

Early bacteraemia and bleeding are potential hazards of (intra-abdominal) use of rtPA[15,27-29]. To our surprise, bacteraemia was not observed in experiment 1. Therefore, we repeated the experiment with a 10,000 fold higher dose of *E. coli*. In that experiment, *E. coli* bacteraemia was observed earlier indeed in the rtPA treated group. Rotstein and associates demonstrated a correlation between rtPA, early *E. coli* bacteraemia, and increased mortality rates[15]. This is not corroborated by the results of our study. Although early bacteraemia was present more frequently in the rtPA group in experiment 2, we did not find a relation between mortality and bacteraemia in either experiment 1 or 2. Nevertheless, Rotstein and associates showed a marked reduction of mortality rates when bacteraemia was avoided by systemic use of antibiotics in their experiments[15]. In concordance with others, we did not observe an increased incidence of bleeding in the abdomen or elsewhere associated with the use of rtPA[27,30]. This is explained most likely by the low tPA activity in the plasma, as was previously discussed.

Recombinant tissue plasminogen activator administered into the abdominal cavity in rats with generalized peritonitis reduces the number of intra-abdominal abscesses. However, early bacteraemia and increased mortality rates are serious drawbacks of the intra-abdominal use of rtPA in this rat model.

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CHAPTER 13

GENTAMICIN REDUCES BACTERAEMIA AND MORTALITY ASSOCIATED WITH THE TREATMENT OF EXPERIMENTAL PERITONITIS WITH RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR

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▲INTRODUCTION

Intra-abdominal abscesses are an important cause of morbidity and mortality in patients with generalized peritonitis[1]. Formation of intra-abdominal abscesses is mediated by fibrin clots, which are colonized by bacteria[2]. Several experimental studies have been attempted to prevent the formation of intra-abdominal abscesses by inhibition of fibrin formation with heparin or by activation of fibrinolysis with plasminogen activators[3,4]. In a previous experiment, we demonstrated a reduction of intra-abdominal abscesses with the use of a viscous gel that contained recombinant tissue plasminogen activator (rtPA) in rats with generalized peritonitis[5]. However, rats treated with rtPA had an increased mortality rate, and early *Escherichia coli* (*E. coli*) bacteraemia was observed more frequently, probably as a result of early degradation of fibrin. In the present study, it was determined whether or not mortality and bacteraemia could be prevented by the administration of gentamicin in rats with generalized peritonitis treated with rtPA intraperitoneally.

▲MATERIALS AND METHODS

Design of the study

Experiment 1

Male Wistar rats, weighing between 255 and 290 g were used. Generalized peritonitis was induced by intraperitoneal injection of 2 ml faecal suspension containing 10^9 colony forming units (cfu)/ml *E. coli* and 10^4 cfu/ml *Bacteroides fragilis* (*B. fragilis*). After one hour, a midline laparotomy was performed under general anaesthesia (halothane-nitrous oxide-oxygen mixture). The abdomen was debrided (including partial omentectomy) and rinsed with normal saline solution. Before closure of the abdomen, the rats received either 2.5 ml methyl hydroxy propyl cellulose (MHPC) gel (Clinical Pharmacy, Groningen, The Netherlands) (n=14) or 2.5 ml MHPC gel containing 0.5 mg/ml human rtPA (Boehringer Ingelheim, Alkmaar, The Netherlands) (n=20) intraperitoneally. MHPC gel was used as a vehicle. After operation, 5 ml of normal saline solution were administered subcutaneously for resuscitation. At three hours after induction of peritonitis,

one-half of the rats in each group received gentamicin sulphate, 6 mg/kg body weight, intramuscularly (IM) in a single dose. Thus, four experimental groups were formed: MHPC gel alone (M), MHPC gel *plus* rtPA (M-tPA), MHPC gel *plus* gentamicin (M-G), and MHPC gel *plus* rtPA *plus* gentamicin (M-tPA-G). At one, three, six, 12 and 24 hours after inoculation, blood samples (0.45 ml) were taken by cardiac puncture while rats were under general anaesthesia to assess bacteraemia. Lost blood volume was compensated by subcutaneous administration of 5 ml of normal saline solution. Five days after inoculation, the surviving rats were sacrificed by intracardiac pentobarbital (Nembutal®, Abbott Laboratories, North Chicago, USA) injection and inspected for intra-abdominal abscess formation.

Experiment 2

In a similar experiment, published previously[5], we inoculated 10^4 instead of 10^8 cfu/ml *E. coli*. It was found that mortality rates in rats treated with rtPA were significantly higher but bacteraemia was not observed. In the present study, this experiment was repeated including a group of rats receiving gentamicin IM.

The same model of intra-abdominal infection and treatment was used as already described. Faecal suspension contained 10^4 cfu/ml *E. coli* and 10^4 cfu/ml *B. fragilis*. At laparotomy, the rats received either 2.5 ml of MHPC gel (n=16) or 2.5 ml of MHPC gel containing 0.5 mg/ml rtPA (n=24). Half of the rats in each group received gentamicin sulphate (6 mg/kg body weight IM, three hours after inoculation). Thus, analogous experimental groups to those in experiment 1 were obtained. Blood cultures were taken at three, six, 12 and 24 hours after inoculation. Five days after inoculation, the abdomens of surviving rats were reopened under sterile conditions and abscesses were counted. If present, abscesses on different spots were excised for culture. During the experiments, the rats had free access to water and standard rat chow (MH-B 1010, Hope Farms, Woerden, The Netherlands).

Bacterial faecal suspension

E. coli strain American Type Culture Collection (ATCC) 25922 and *B. fragilis* from a clinical isolate were used. Bacteria were cultured according to standard methods. The mean inhibitory concentration of gentamicin for this *E. coli* strain was 0.5 mg/l as measured by the E-test (AB Biodisk, Solna, Sweden).

Fresh faeces of Wistar rats were prepared as described previously[5]. Prior to intraperitoneal injection, the sterile faecal suspension was vigorously mixed with the bacterial suspensions.

Cultures of abscesses

The excised abscesses were cultured semiquantitatively using standard aerobic and anaerobic bacteriologic techniques.

Blood cultures

Blood (0.45 ml) was collected in sterile tubes containing 0.05 ml buffered citrate. Serial dilutions in Brain Heart Infusion broth were made and plated onto both aerobic (MacConkey, blood agar; Oxoid, Hampshire, United Kingdom) and anaerobic (Bacteroides bile esculine agar, Brucella blood agar; Becton-Dickinson, Baltimore, USA) plates. After 24 and 48 hours of incubation at 37°C bacteria were counted and identified. Bacterial concentration was expressed in ^{10}log cfu/ml whole blood.

Statistics

Statistical analysis was performed using chi-square test, Yates and Cochran or Kruskall Wallis rank analysis, when appropriate. When the p-value was less than 0.05, a difference between groups was considered significant.

▲RESULTS

Experiment 1

At one hour after inoculation none of the rats had bacteraemia. At 12 hours after inoculation, all rats in group M and M-tPA had bacteraemia, and all died within 24 hours. Bacteraemia occurred earlier in rats in group M-tPA in comparison with those in group M, as reflected by a significantly ($p<0.05$) higher number of rats with bacteraemia at six hours after inoculation (Figure 1). Gentamicin significantly reduced the number

of rats with bacteraemia: in the M-tPA-G group at six and 12 hours and in the M-G group at 12 hours after inoculation. Also, mortality was significantly ($p<0.05$) reduced by gentamicin in these groups of rats (Figure 2).

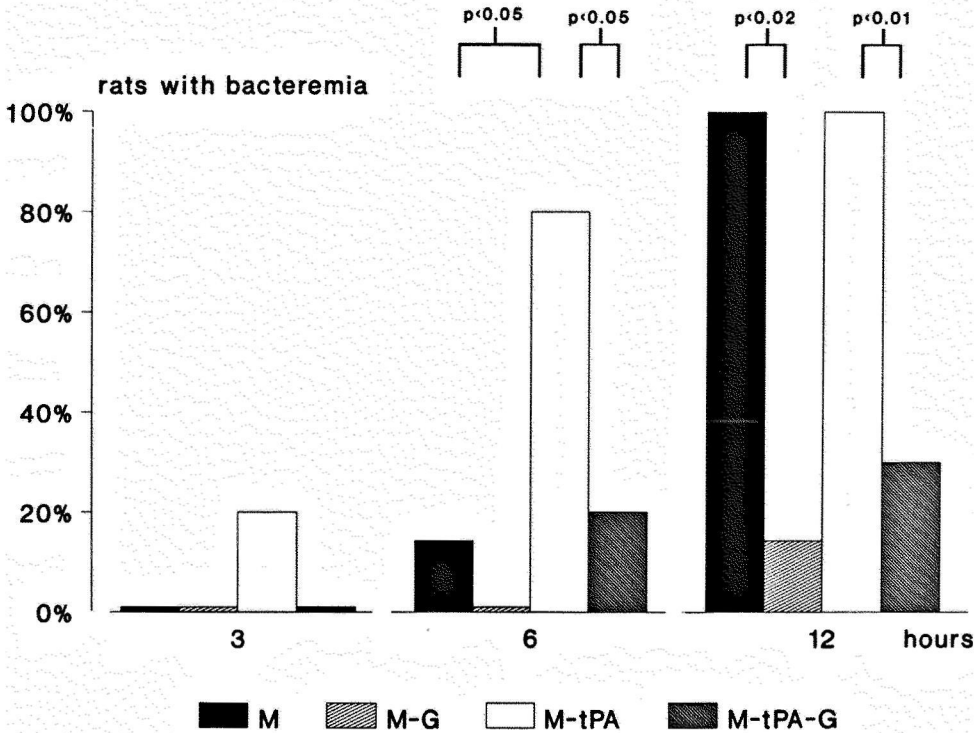

Figure 1. Percentage of rats with bacteraemia at three, six and 12 hours after inoculation of 2 ml sterile faeces contaminated with 10^8 cfu per ml *E. coli* and 10^4 cfu per ml *B. fragilis*. At six hours there was a significant effect of gentamicin in the M-tPA-G group and at 12 hours in both groups. At six hours significantly ($p<0.05$) more rats in group M-tPA than in group M had bacteraemia.

At 12 hours after inoculation, blood cultures revealed a median bacterial concentration of 10^5, ranging from 10^2 to 10^7, in rats in group M and M-tPA. The bacterial concentration was significantly ($p<0.05$) reduced in rats treated with gentamicin to a median concen-

tration of zero (range, zero to 10^7). However, the bacterial concentration in the one rat in group M-G and the three rats in group M-tPA-G that had bacteraemia did not differ significantly from those in group M and M-tPA: 10^4 (10^2-10^5) compared with 10^5 (10^2-10^7). Only *E. coli* (ATCC 25922) was isolated from the blood. At 24 hours after inoculation, all blood cultures available from surviving rats revealed no growth. Three of the six surviving rats in group M-tPA-G were free of intra-abdominal abscesses at day 5, whereas all five surviving rats in group M-G had multiple intra-abdominal abscesses.

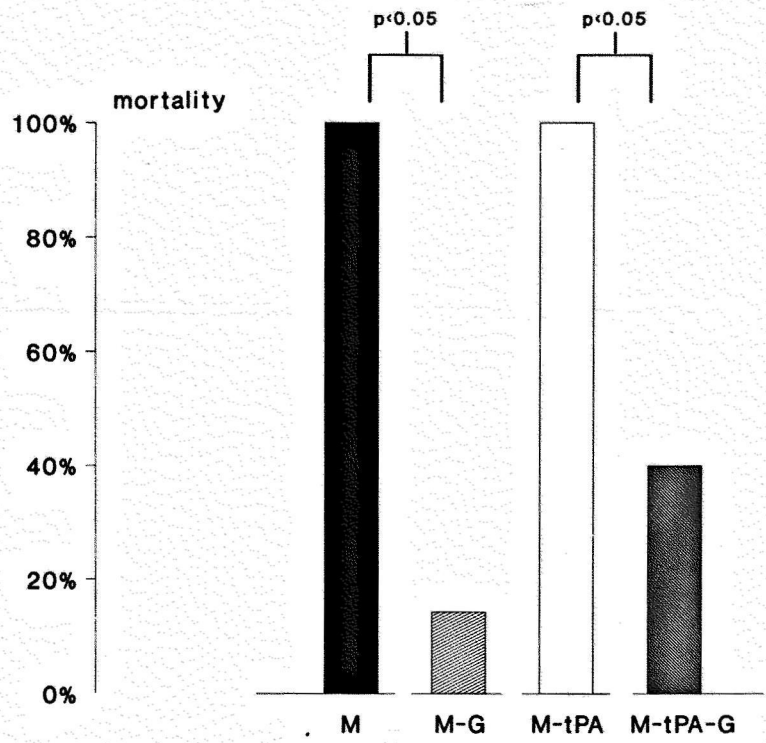

Figure 2. Mortality rates in rats after inoculation of sterile faeces contaminated with 10^8 *E. coli* and 10^4 *B. fragilis*. Mortality is significantly ($p<0.05$) reduced by gentamicin.

Experiment 2

When inoculated with 10^4 *E. coli*, five out of 12 rats (42%) in group M-tPA died compared to zero out of eight rats (0%) in group M. Rats died between 12 and 24 hours after inoculation and none of them had abscesses at autopsy. Positive blood cultures were not found in any rat during this experiment. Surprisingly, gentamicin prevented mortality: 0/12 in group M-tPA-G compared with 5/12 in group M-tPA; $p<0.05$. None of the eight rats in group M-G died.

Five days after inoculation, intra-abdominal abscesses were found in all rats in group M and M-G, in six of seven surviving rats in group M-tPA, and in 11 of 12 rats in group M-tPA-G. In four rats (two in group M-G, two in group M-tPA-G) no bacteria were isolated from the excised abscesses. In rats with positive cultures of intra-abdominal abscesses and treated with rtPA, the median number of abscesses was significantly lower ($p<0.01$) than in those not treated with rtPA: 3 (range, 1-4) in group M-tPA and 4 (range, 1-8) in group M-tPA-G compared with 11 (range, 8-15) in group M and 10 (range, 7-13) in group M-G. Gentamicin did not influence the number of abscesses.

E. coli ATCC 25922 was cultured from abscesses in most cases (Table 1). In contrast, *B. fragilis* was not found. Other species seemed to be more frequently isolated from abscesses in rats treated with gentamicin compared with those not treated with gentamicin.

Table 1. Bacteriology of intra-abdominal abscesses in surviving rats with positive cultures

group	number of rats with positive cultures	number of rats with *E. coli* ATCC 25922 (%)	number of rats with other species* (%)
M	8	7 (88)	4 (50)
M-G	6	4 (67)	6 (100)
M-tPA	6	6 (100)	1 (17)
M-tPA-G	9	7 (78)	6 (67)

*other species are *Enterococci*, *Staphylococci*, *Proteus*, and *Candida*, which were not sensitive to gentamicin

▲DISCUSSION

The present study demonstrates that antibiotic treatment markedly reduces early bacteraemia and reduces the mortality rate in rats with generalized peritonitis that are treated with rtPA to prevent the formation of residual abscesses.
Fibrin entraps bacteria, thus preventing early bacteraemia and mortality in generalized peritonitis[2,6]. However, these colonized fibrin clots are the nidus for intra-abdominal abscesses. It is expected that intra-abdominal application of rtPA to prevent these abscesses will cause early bacteraemia. In concordance with previous findings, early bacteraemia occurred in association with the use of rtPA[4,5].
A single dose of 6 mg/kg gentamicin sulphate, administered intramuscularly three hours after onset of peritonitis, reduced bacteraemia and mortality rates in experiment 1, and the mortality rates in experiment 2. rtPA appeared to be the main factor reducing abscess formation in contrast to gentamicin. Several authors have reported the inability of antibiotics to reduce abscess formation[6-8]. Hau and associates have demonstrated, using a fibrin clot model, that gentamicin like several other antibiotics does not influence abscess formation mainly because inhibitory levels within the fibrin clot are not reached[7]. However, in the same model a reduction of colony count per abscess by gentamicin sulphate has been reported[8]. We did not quantitate bacteria in abscesses. Qualitatively, the use of gentamicin seemed to have altered the bacterial flora of the abscesses. The absence of *B. fragilis* in the abscesses was remarkable. This might be due to the peritonitis model used, as was pointed out previously[5].
From this and previous studies[4,5,9] it emerges that intra-abdominal administration of rtPA may be a valuable adjunct to the standard treatment of patients with generalized peritonitis in order to prevent intra-abdominal abscess formation. Antibiotics directed against the causal bacteria are an essential part of this treatment. With the use of appropriate antibiotics, the risk of bacteraemia associated with the use of rtPA in the clinical situation is expected to be minor. Bleeding, another potential risk of the use of rtPA clinically, has so far not been observed in experiments wherein rtPA has been administered into the abdominal cavity[5,10].

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CHAPTER 14

GENERAL DISCUSSION

▲INTRODUCTION

Generalized peritonitis, caused by a perforated viscus or anastomotic leakage, is a life-threatening illness. With surgical intervention, antibiotic therapy and supportive techniques for the care of patients with serious intra-abdominal infection, mortality has decreased from 90% early this century to 25% in more recent years[1]. Despite these advances, mortality from several forms of intra-abdominal infection remains high. For example, patients with large bowel perforation, anastomotic leakage and pancreatic necrosis have mortality rates, varying from 20% to 60%[2]. Many of these patients suffer from persistent or recurrent intra-abdominal infection and eventually die due to multiple organ dysfunction syndrome[3].

In the past twenty years, new operative techniques, such as "radical debridement", continuous postoperative peritoneal lavage and "planned relaparotomy" have been introduced to treat patients with severe intra-abdominal infection[4-6]. These techniques have a common aim: to eliminate infection potentiating material, such as bacteria, necrosis and fibrin from the abdominal cavity as radical and as soon as possible, in order to prevent residual or recurrent infection.

In this thesis, the results of "planned relaparotomy" to treat patients with generalized peritonitis due to perforation of the colon or anastomotic leakage (Chapter 6) and patients with infected pancreatic necrosis (Chapter 7), have been described.

The goal of this aggressive treatment was achieved. In the majority of patients intra-abdominal infection was controlled effectively and only 3% of the patients developed an intra-abdominal abscess. A major drawback of "planned relaparotomy" was the high frequency of intra-abdominal complications. The nature of these complications, such as bleeding and bowel perforation, strongly suggests repeated mechanical removal of fibrin and necrosis to be a causal factor.

The adverse effect of repeated mechanical removal of fibrin and debris from the abdominal cavity has stimulated our search for an alternative to remove fibrin from the abdominal cavity, in order to prevent residual intra-abdominal abscess formation. Enzymatic removal of fibrin, by increasing the fibrinolytic activity in the abdominal cavity, seemed us to be a good alternative.

In this thesis the following objectives were addressed:

1. What is the influence of intra-abdominal infection on the coagulation cascade and the fibrinolytic system in the abdominal cavity (Chapter 8 and 9)?
2. What is the effect of enhancement of fibrinolytic activity on intra-abdominal abscess formation after peritonitis (Chapter 10, 11, 12 and 13)?

1. The influence of intra-abdominal infection on fibrin formation and fibrinolysis in the abdominal cavity

Intra-abdominal inflammatory processes result in the exudation of protein-rich fluid in the abdominal cavity. In this fluid fibrin is formed through activation of the coagulation cascade. These fibrin deposits are degraded by the fibrinolytic system in the peritoneal cavity. When this system fails, fibrinous adhesions are invaded by fibroblasts, and are transformed into permanent fibrous tissue within five days after the insult.

In the peritoneal fluid of our patients with peritonitis the coagulation cascade was found to be activated. It is assumed that the coagulation cascade is activated via the extrinsic pathway, which is initiated by tissue factor (TF)[7]. This assumption is based on the fact that TF is expressed in vitro on the surfaces of cells, involved in peritoneal inflammation, upon stimulation with inflammatory mediators[8]. Furthermore, recent studies in healthy volunteers and non-human primates revealed that the activation of coagulation in plasma by administration of endotoxin or tumor necrosis factor (TNF) is completely TF pathway dependent[9,10]. However, the intrinsic pathway may also been involved in intra-abdominal fibrin formation, since activators of this pathway, such as prekallikrein, have been demonstrated in the peritoneal fluid of patients with peritonitis[11].
We also have demonstrated activation of the fibrinolytic system in combination with high concentrations of plasminogen activator inhibitor (PAI) in the peritoneal fluid of both rats and humans with peritonitis.
It seems that the balance between coagulation and fibrinolysis in the abdominal cavity is disturbed following intra-abdominal infection. This disturbance is probably responsible for persistence of intra-abdominal fibrin and subsequent adhesion and abscess formation. However, the degree of disturbance in the balance between coagulation and fibrinolysis may vary in time during intra-abdominal infection and it may be possible that the balance is rapidly restored after elimination of the infectious focus. Thus, it is important to know the sequential changes of coagulation and fibrinolysis in the abdominal cavity during

infection and after removal of the infectious source.

To our knowledge, there are no longitudinal studies of the coagulation response in the peritoneal fluid to bacterial peritonitis. However, it is obvious that fibrin will be formed in the abdominal cavity as long as the infectious focus remains in situ.
Stimulation of the coagulation process may continue for some time after elimination of the source of infection, since the inflammatory response will diminish only slowly.
More is known about sequential changes in the fibrinolytic response to bacterial peritonitis. Recently, Vipond et al. measured the time course of alteration in plasminogen activating activity (PAA, resultant of activation and inhibition of fibrinolysis) in biopsies of the peritoneum in an animal model of mild bacterial peritonitis[12]. They demonstrated a pronounced reduction of PAA in the first 12 hours after inoculation followed by a rise of PAA and return to baseline levels at one week. The initial drop of PAA was attributed to increased concentrations of PAI, whereas the rise of PAA thereafter was related to increased concentrations of tissue-type plasminogen activator (tPA).
Most studies have shown that the increased PAI activity is mainly responsible for the initial depression of fibrinolytic activity during peritonitis[12-14]. We have demonstrated not only an initial increase in PAI activity in the peritoneal fluid of rats with peritonitis, but persistently elevated levels of PAI activity for 5 to 8 days after the initial insult[1-5]. This persistent elevation of PAI might be attributed to the presence of residual intra-abdominal infection in our experimental model. Others found a reduction of peritoneal fibrinolytic activity, which was due to increased release of plasminogen activator inhibitors, for more than 4 days after abrasion of a great part of the parietal peritoneum[16].
The regeneration of fibrinolytic activity in the peritoneum coincides with the timing of healing of the peritoneum. Histological studies of peritoneum after mild ischaemic or mechanic injury show complete healing within 7 days[17-19]. The timing of healing of the peritoneum might depend on the severity of the initial injury[16]. In our experiment of rats with faecal peritonitis the peritoneum still was not healed 8 days after bacterial injury[15]. This delay in peritoneal healing may not only be explained by the severity of peritoneal injury but by the repeated laparotomies and the presence of residual intra-abdominal abscesses in these rats.

The effect of removal of the infectious focus on fibrinolytic activity has not been investigated

sofar. There is scarce information on the effect of surgery itself on the fibrinolytic activity. Recently, a biphasic fibrinolytic response of human peritoneal fluid to elective abdominal surgery was found by Scott-Coombes et al.: an early reduction of PAA secondary to a reduction in tPA levels in the first 24 hours after surgery and subsequent loss of PAA due to an increase in PAI-1 and PAI-2 concentrations in the following 24 hours[20]. The influence of surgery on fibrinolytic activity of rabbit peritoneal macrophages has been described by Orita et al[21]. They have demonstrated a reduction in PAA of macrophages recovered from the peritoneal fluid on the first and second postoperative day, followed by an increase in PAA reaching peak values on the 14th postoperative day. They have not determined if a rise in tPA or urokinase-type plasminogen activator (uPA) or a decline in PAI-2 was responsible for the rise in PAA after the second postoperative day.

Based on the above mentioned it is hypothesized that patients with severe intra-abdominal infection suffer from a prolonged activation of the coagulation cascade in the abdominal cavity, a prolonged depression of fibrinolytic activity because of persistently elevated PAI levels, and a delay in peritoneal healing because of severe peritoneal damage. Repeated laparotomies in these patients might contribute to continuous activation of coagulation and might jeopardize peritoneal healing and regeneration of fibrinolytic activity.

The sequential changes in coagulation and fibrinolysis in the abdominal cavity during intra-abdominal infection and after surgical elimination of the infectious focus need to be further elucidated. Moreover, the contribution of various cells such as mesothelial cells, endothelial cells and peritoneal macrophages to the coagulation and fibrinolytic processes is largely unknown. Future studies should address these topics in order to fully understand why in some circumstances fibrin deposits persist in the abdominal cavity and cause intra-abdominal abscesses.

2. The effect of enhancement of fibrinolytic activity on intra-abdominal abscess formation

Fibrin deposition in the abdominal cavity may be prevented by inhibition of the intraperitoneal coagulation cascade and/or enhancement of the intraperitoneal fibrinolytic activity.

Inhibition of the coagulation cascade

Heparin has shown to be successful in several experimental studies, reducing intra-abdominal abscess formation and preventing mortality from peritonitis[22-25]. Major drawbacks of heparin, however, are the unpredictable anticoagulant effect and the risk of bleeding. More selective antithrombotic agents, such as low molecular weight heparins, hirudin, specific factor Xa inhibitor and anti-TF have recently been studied in experimental models of arterial and venous thrombosis and in experimental endotoxaemia[26-29]. These agents may not only be useful in attenuating intra-abdominal fibrin formation, but also to unravel the pathways involved in intra-abdominal coagulation.

Enhancement of the fibrinolytic activity

We have used human recombinant tPA (rtPA) to enhance the intra-abdominal fibrinolytic activity, in order to prevent intra-abdominal abscess formation in experimental peritonitis[30]. It was hypothesized that rtPA would have a pronounced local effect because of its high affinity for fibrin, and a minimal systemic fibrinolytic effect, because of high circulating PAI levels in peritonitis. Our study on the pharmacokinetics of rtPA and those on prevention of experimental intra-abdominal abscesses seem to support this hypothesis.

Administration of rtPA early in the course of peritonitis successfully reduced intra-abdominal abscess rate. In this period endogenous PAI activity still was relatively low, which may have contributed to this success. Later in the course of peritonitis, treatment with rtPA probably is less-effective due to inhibition by PAI and to resistance of fibrin deposits to lysis by cellular invasion and collagen production. This may have implications for the use of rtPA in the clinical situation, as in patients the infectious process may be present for some time before institution of therapy.

In patients with generalized bacterial peritonitis rtPA may increase the risk of bacteraemia. This risk probably is low, since most patients with generalized peritonitis are treated systemically with (broad-spectrum) antibiotics for several days. Local efficacy of antibiotics is expected even to be increased, as bacteria are no longer protected by fibrin in the abdominal cavity.

Another risk of the use of rtPA in patients with peritonitis is bleeding. In our animal experiments this complication was not observed, most likely due to high concentrations of PAI in the plasma during peritonitis. In patients with peritonitis we did not find elevated plasma PAI concentrations. This might be explained by the local character of the infectious

process in the majority of our patients. Paramo et al. demonstrated strongly elevated plasma levels of PAI in patients with generalized infectious processes[31].

Fibrinolytic activity in the abdominal cavity may also be influenced by inhibition of PAI with, for example, PAI-1 neutralizing monoclonal antibody. Recently, it has been demonstrated that PAI-1 monoclonal antibody promotes endogenous thrombolysis in experimental models of venous and arterial thrombosis[32]. The use of antagonists towards plasminogen activator inhibitors may not only be of therapeutic interest but to study the non-inhibited endogenous tPA and uPA activity in the abdominal cavity during peritoneal healing.

Inhibition of inflammatory cytokines is a less specific approach to influence fibrinolytic activity in order to prevent intra-abdominal abscess formation. Anti-TNF antibodies have shown to abolish plasma PAI-1 activity in experimental endotoxaemia[33]. However, tPA activity was also completely blocked. Similar results were obtained with the use of pentoxifylline[33]. In a study of Chalkiadakis et al., pentoxifylline reduced the development of abscesses in the abdominal cavity of rats with peritonitis, which effect was attributed to increased fibrinolytic activity[34].

Modulation of coagulation and/or fibrinolysis in the abdominal cavity by the intra-abdominal administration of drugs may be a useful adjunct to surgery in patients with generalized peritonitis and a high risk of developing a residual intra-abdominal infection. In combination with appropriate antibiotics infection may be cleared very rapidly, preventing the deleterious local and systemic effects of persistent intra-abdominal infection.

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SUMMARY

Patients with severe intra-abdominal infection, caused for example by perforation of the colon, leakage of intestinal anastomoses or pancreatic necrosis, have a poor prognosis. Conventional operative therapy in this condition frequently results in persistent or recurrent intra-abdominal infection.
Persistence of fibrin deposits in the abdominal cavity is associated with development of residual intra-abdominal infection. Removal of fibrin may beneficially influence residual infection rate and patient outcome. New operative methods, characterized by meticulous removal of all infection potentiating material, such as fibrin, from the abdominal cavity have reduced the incidence of persistent or recurrent infection. However, morbidity associated with these methods is high and improved survival is not evident.
In this thesis we focused on fibrin formation and fibrin degradation in the abdominal cavity during peritonitis and the effect of enzymatical removal of fibrin on residual intra-abdominal infection, in a search for adjuvant treatment to surgery in patients with severe intra-abdominal infection.

Chapter 2 describes the anatomy and (patho)physiology of the abdominal cavity. Local host defenses are described, including the formation of fibrin in the abdominal cavity. The dualistic character of fibrin is outlined: on the one hand sequestration of bacteria, preventing bacteraemia, on the other hand impairment of other local defenses, which may lead to residual intra-abdominal infection.

In **Chapter 3** the definitions and aetiology of intra-abdominal infections are outlined. Most frequently used are the terms: primary, secondary and tertiary peritonitis. Secondary peritonitis, which is caused by perforation or transmural necrosis of the gastrointestinal tract is most important for surgeons. In this condition a polymicrobial flora of gut derived bacteria will be isolated from the abdominal cavity in most cases.

Chapter 4 focuses on the surgical treatment of intra-abdominal infection. Elimination of the source of infection and debridement and lavage of the abdominal cavity are the cornerstones of operative treatment. Various surgical options of treatment after the initial operation in patients with severe intra-abdominal infection are discussed. The optimal surgical strategy is still under debate.

In **Chapter 5** the coagulation cascade, the fibrinolytic system, intra-abdominal coagulation

and fibrinolytic processes in peritonitis and the possibilities to modulate intra-abdominal fibrin deposition are outlined. During infection, intra-abdominal coagulation is enhanced and fibrinolytic activity in the peritoneum is depressed. This latter is held responsible for persistence of fibrin deposits in the abdominal cavity.
There are scarce data on successful modulation of intra-abdominal coagulation and fibrinolysis in experimental peritonitis; clinical data are lacking.

In **Chapter 6** an analysis of the intra-abdominal complications of "planned relaparotomy" in 24 patients with severe generalized peritonitis is reported and an attempt has been made to define when to stop relaparotomies. Intra-abdominal complications occurred in 71% of the patients, more frequently in nonsurvivors than in survivors, and correlated strongly with the number of planned relaparotomies.
Control of intra-abdominal infection (less than 10^3 colony forming units (cfu) per ml microorganisms) was achieved after three relaparotomies in the majority of patients. Organ dysfunction, as measured by the MOF score, remained unchanged in the first week of "planned relaparotomy".
It is concluded that "planned relaparotomy" introduces pronounced morbidity and does not reverse organ dysfunction at the short run. The - less than 10^3 cfu/ml - criteria may be useful for the decision to cease relaparotomies.

In **Chapter 7** we report on early and long term results of necrosectomy, planned re-explorations and "open" drainage in the management of 10 patients with infected pancreatic necrosis. Three patients died because of multiple organ failure. In half of the patients complications such as intra-abdominal haemorrhage, necrosis of the transverse colon and enterocutaneous fistula were encountered. None of the patients developed a residual intra-abdominal abscess.
One patient developed steatorrhoea, another developed insulin dependent diabetes mellitus during follow-up. All non-diabetic patients had an impaired glucose tolerance and significantly elevated glucagon and insulin serum levels compared to matched healthy volunteers.
It is concluded that this treatment method prevents residual intra-abdominal abscesses in patients with infected pancreatic necrosis but is associated with pronounced morbidity. Long term glucose metabolism is disturbed, as demonstrated by impaired glucose tolerance and elevated serum insulin and glucagon levels.

Chapter 8 describes the coagulation and fibrinolytic responses of the peritoneal fluid and plasma to bacterial peritonitis in humans. In 25 patients with bacterial peritonitis and seven control patients, thrombin-antithrombine III (TAT) complex (measure of activation of coagulation), tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), plasminogen activator inhibitor (PAI), plasmin-α_2-antiplasmin (PAP) complex (measure of stimulation of fibrinolysis) and fibrin degradation products (FbDP) were measured in the peritoneal fluid and plasma.
TAT complex was significantly higher in the peritoneal fluid of patients with peritonitis than in control patients. Peritoneal fluid concentrations of tPA and uPA in peritonitis were increased by a factor 65 and 10, respectively; concentrations of PAI (inhibitor of tPA and uPA) by a factor 400. Despite high intra-abdominal PAI concentrations, intra-abdominal fibrinolysis was stimulated in peritonitis patients in comparison with controls, as reflected by significantly higher PAP complex and FbDP. Plasma concentrations of uPA and FbDP were significantly higher in peritonitis patients, as compared with controls. No correlations were found between plasma and peritoneal fluid levels.
It is concluded that in bacterial peritonitis, both intra-abdominal coagulation and fibrinolysis are stimulated. However, due to inhibition of plasminogen activators, fibrinolytic activity may not meet its demand.

In **Chapter 9** the activities of tPA and PAI were measured in the peritoneal fluid of rats with faecal peritonitis (intra-abdominal injection of sterilized faeces contaminated with 10^4 cfu/ml *Escherichia coli* (*E. coli*) and *Bacteroides fragilis* (*B. fragilis*) to determine the cause of persistence of intra-abdominal fibrin with subsequent adhesion and abscess formation. These activities were correlated with the extent of peritoneal damage.
Activity of tPA was low during the study period of 8 days. In rats with peritonitis, tPA activity was higher than in controls. The activity of PAI in rats with peritonitis was significantly increased compared with controls during the whole study period. Histological signs of damage to the peritoneum were similar in rats with peritonitis and controls. There was no correlation between the extent of peritoneal damage and tPA or PAI activity.
The increased activity of PAI in the peritoneal fluid of rats with faecal peritonitis may be the main cause of reduced fibrinolysis in the abdominal cavity. Activities of tPA and PAI may originate not only from the mesothelium but from other sources.

Chapter 10 provides data on the in vitro interaction between human recombinant tPA (rtPA) and the rat fibrinolytic system. There were several cross-reactivities between human rtPA and rat factors, as deduced from lysis of rat clots by human rtPA, plasminaemic fibrinogen and fibrin degradation in rat plasma by human rtPA, and inhibition of human rtPA by rat PAI. However, rat plasma clots seemed to be more resistant to lysis by human rtPA in comparison with human plasma clots. Moreover, the assay to detect fibrin degradation products was less sensitive to rat FbDP than human FbDP. Streptokinase was unable to lyse rat plasma clots.
The interaction between human rtPA and components of the rat fibrinolytic system makes the rat a useful animal for studies on modulation of fibrinolytic activity by human rtPA in peritonitis.

Chapter 11 describes the pharmacokinetics of human rtPA, when administered intraperitoneally in rats with faecal peritonitis. In the first experiment the endogenous tPA and PAI activity in the peritoneal fluid and plasma were measured in rats with faecal peritonitis. In the second experiment tPA antigen and tPA activity were measured in both compartments after intra-abdominal administration of a single dose of either 0.5 mg/ml or 2.0 mg/ml human rtPA to rats with peritonitis.
Endogenous tPA activity in the peritoneal fluid was only slightly elevated, whereas endogenous PAI activity showed a strong increase with a peak at 16 hours. In plasma only PAI activity was increased.
Both doses of human rtPA substantially enhanced fibrinolytic activity of the peritoneal fluid up to 24 hours. However, tPA activity measured in the peritoneal fluid of rats with peritonitis was lower than that calculated from the measured tPA antigen levels. Plasma tPA activity was low at both doses. Use of a gel in comparison with Ringer's lactate solution, as a solvent of rtPA, lowered the plasma concentration of tPA antigen.
A single dose of 0.5 mg/ml or 2.0 mg/ml human rtPA will raise fibrinolytic activity in the abdominal cavity up to 24 hours. High concentrations of endogenous PAI in the peritoneal fluid may adversely effect intra-abdominal use of human rtPA in peritonitis. The risk of bleeding, associated with the (intra-abdominal) use of rtPA in peritonitis, is probably low.

In **Chapter 12** the effect of increasing the intra-abdominal fibrinolytic activity on abscess formation by intra-abdominal administration of human rtPA in rats with faecal peritonitis

is reported (the peritonitis model has been described in Chapter 9).
Rats, treated with rtPA dissolved in methyl hydroxy propyl cellulose (MHPC) gel (0.5 mg per ml), which was used as a vehicle, had significantly less intra-abdominal abscesses than rats in the control groups, given either Ringer's lactate solution or MHPC gel alone. Other than *E. coli*, cultures of abscesses revealed species originating from the intestine, demonstrating bacterial translocation. The mortality rate was significantly higher in rats treated with rtPA as compared with controls, which was surprising considering the absence of bacteraemia. By challenging the rats with a higher dose of *E. coli* in the inoculum (10^8 cfu/ml), early bacteraemia was observed in the rats treated with rtPA, not related to increased mortality rates. Intra-abdominal use of rtPA was not associated with an increased incidence of bleeding events.
Recombinant tissue-type plasminogen activator prevents abscess formation in rats with faecal peritonitis. However, early bacteraemia and increased mortality rates are serious drawbacks of the intra-abdominal use of rtPA in this rat model.

In **Chapter 13** the effect of gentamicin on mortality and bacteraemia, which are induced by intraperitoneal administration of rtPA in rats with faecal peritonitis, as described in the previous chapter, is studied.
Gentamicin significantly reduced the number of rats with bacteraemia, the bacterial concentration in the blood, and mortality at the high dose *E.coli* in the inoculum (10^8 cfu/ml). Although none of the rats at the low dose *E. coli* in the inoculum (10^4 cfu/ml) developed bacteraemia, gentamicin prevented mortality associated with the use of rtPA. The number of abscesses in animals treated with rtPA was significantly lower than in those not treated with rtPA. Gentamicin did not influence the number of abscesses.
It is concluded that gentamicin reduces bacteraemia and mortality in rats with faecal peritonitis treated with rtPA, that is administered intraperitoneally to prevent intra-abdominal abscess formation.

Chapter 14 contains the general discussion. Both the coagulation cascade and the fibrinolytic system in the abdominal cavity are stimulated, as a response to intra-abdominal infection. However, due to a concomitant increase of inhibitors of the fibrinolytic system, fibrinolytic activity may not meet its demand. As a result fibrin may persist in the abdominal cavity, become organized and may promote residual intra-abdominal

infection. Increasing the fibrinolytic activity in the abdominal cavity by means of rtPA in combination with antibiotic treatment, is effective and safe in the prevention of residual intra-abdominal infection in rats with faecal peritonitis.
Further longitudinal studies are needed to elucidate the changes in coagulation and fibrinolysis in the abdominal cavity during peritonitis and after elimination of the infectious focus, in order to understand why in some circumstances fibrin persists and promotes residual intra-abdominal infection, and fibrinolytic therapy may be indicated.

SAMENVATTING

De prognose van patiënten met een ernstige intra-abdominale infectie, veroorzaakt door bijvoorbeeld een colonperforatie, lekkage van een intestinale anastomose of pancreas necrose is slecht. De behandeling van deze patiënten is niet altijd succesvol met als gevolg aanwezig blijven of het opnieuw optreden van intra-abdominale infectie. Er is een verband tussen het aanwezig blijven van fibrinedeposities in de buikholte en de ontwikkeling van restinfectie. Verwijdering van fibrine zou het percentage restinfectie en daarmee de prognose van de patiënt gunstig kunnen beïnvloeden. Nieuwe operatieve methoden, die worden gekenmerkt door het zorgvuldig uit de buikholte verwijderen van al het infectie onderhoudende materiaal -zoals fibrine-, hebben de frequentie van optreden van restinfectie gereduceerd. De morbiditeit van deze methoden is echter hoog en een gunstig effect op de overleving is niet duidelijk aangetoond. De fibrinevorming en -afbraak in de buikholte tijdens peritonitis en het effect van, op enzymatische wijze, verwijderen van fibrine op het optreden van restinfectie in de buik, als aanvullende behandeling op de operatie bij patiënten met een ernstige intra-abdominale infectie staat centraal in dit proefschrift.

Hoofdstuk 2 bevat de beschrijving van de anatomie en de (patho)fysiologie van de buikholte. De locale afweermechanismen worden beschreven, waaronder de fibrinevorming in de buikholte. De tweeledige werking van fibrine wordt uiteengezet: enerzijds het sequestreren van bacteriën in de buikholte, waardoor bacteriëmie wordt voorkomen, anderzijds het remmen van andere locale afweer mechanismen, hetgeen kan leiden tot restinfectie in de buik.

In **Hoofdstuk 3** worden de definities en etiologie van intra-abdominale infecties uiteengezet. De termen primaire, secundaire en tertiaire peritonitis worden het meest frequent gebruikt. Secundaire peritonitis, die wordt veroorzaakt door een perforatie of transmurale necrose van de tractus digestivus, is voor chirurgen de belangrijkste vorm van peritonitis. Meestal wordt bij deze vorm een polymicrobiële darmflora uit de buikholte geïsoleerd.

In **Hoofdstuk 4** staat de chirurgische behandelwijze van intra-abdominale infecties centraal. De hoekstenen van de operatieve behandeling zijn het opruimen van de infectiebron, het verwijderen van debris en het spoelen van de buikholte. De diverse chirurgische behandelingsmogelijkheden na de eerste operatie bij patiënten met een

ernstige intra-abdominale infectie worden besproken. De optimale behandelingsstrategie staat nog ter discussie.

In **Hoofdstuk 5** worden achtereenvolgens uiteengezet: de stollingscascade, het fibrinolytische systeem, de stollings- en fibrinolytische processen in de buik tijdens peritonitis en de mogelijkheden om fibrine-afzetting in de buik te beïnvloeden. Tijdens infectie wordt de stolling in de buik geactiveerd en de fibrinolytische activiteit van het peritoneum onderdrukt. Dit laatste wordt verantwoordelijk geacht voor het aanwezig blijven van fibrinedeposities in de vrije buikholte.
Er zijn weinig gegevens over succesvolle beïnvloeding van de stolling en fibrinolyse in de buik tijdens experimentele peritonitis; klinische gegevens ontbreken volledig.

In **Hoofdstuk 6** worden de intra-abdominale complicaties geanalyseerd die optraden bij 24 patiënten met ernstige gegeneraliseerde peritonitis en die zijn behandeld volgens de methode van de "geplande relaparotomie". Tevens wordt getracht te definiëren wanneer de relaparotomieën moeten worden gestaakt. Intra-abdominale complicaties traden op bij 71% van de patiënten en frequenter bij patiënten die overleden. De complicaties stonden duidelijk in relatie met het aantal geplande relaparotomieën. In de meeste patiënten was de intra-abdominale infectie onder controle (minder dan 10^3 kolonie vormende eenheden micro-organismen per ml) na drie relaparotomieën. De mate van orgaanfalen, gemeten door middel van de MOF-score, veranderde niet gedurende de eerste week waarin geplande relaparotomieën werden uitgevoerd.
De conclusie luidt dat de methode van de "geplande relaparotomie" gepaard gaat met een hoge morbiditeit en dat de orgaanfunctie op korte termijn niet verbetert. Het criterium " minder dan 10^3 kolonie vormende eenheden micro-organismen per ml" is misschien van nut voor de beoordeling wanneer de relaparotomieën moeten worden gestaakt.

In **Hoofdstuk 7** doen wij verslag van de vroege en late resultaten van verwijdering van necrose, geplande reëxploraties en open drainage als behandelingsmethode bij 10 patiënten met geïnfecteerde necrose van het pancreas. Drie patiënten stierven ten gevolge van meervoudig orgaanfalen. De helft van de patiënten had abdominale complicaties zoals een bloeding, necrose van het colon transversum en enterocutane fistelvorming. Bij geen van de patiënten werd een restabces in de buik gevonden.

Tijdens de follow-up ontwikkelde één patiënt een steatorrhoe en een andere patiënt een insuline afhankelijke diabetes mellitus. Alle niet-diabetische patiënten hadden op de lange termijn een gestoorde glucose tolerantie, gepaard gaande met significant hogere insuline en glucagon spiegels in het serum in vergelijking met gematchte gezonde vrijwilligers.
Hoewel deze behandelingsmethode restabcessen in de buik voorkomt, gaat zij gepaard met een hoge morbiditeit. Zoals blijkt uit de verminderde glucose tolerantie en de verhoogde insuline en glucagon spiegels is het glucose metabolisme op langere termijn gestoord.

In **Hoofdstuk 8** wordt de activatie beschreven van stolling en fibrinolyse in het buikvocht en het plasma van patiënten met een bacteriële peritonitis. Bij 25 patiënten met een bacteriële peritonitis en zeven controle patiënten werden het trombine-antitrombine III (TAT) complex (maat voor de stollingsactivatie), tissue-type plasminogeen activator (tPA), urokinase-type plasminogeen activator (uPA), plasminogeen activator inhibitor (PAI), plasmine-α_2-antiplasmine (PAP) complex (maat voor de stimulatie van de fibrinolysis) en de fibrine afbraakproducten (FbDP) gemeten in het buikvocht en het plasma.
Het TAT complex in het buikvocht was significant hoger bij de patiënten met een peritonitis dan bij de controle patiënten. De buikvochtconcentraties van tPA en uPA waren 65 respectievelijk 10 maal verhoogd tijdens peritonitis; de PAI (remmer van tPA en uPA) concentratie was 400 maal verhoogd. Ondanks de hoge PAI spiegels was er, gezien de hogere PAP complex en FbDP concentraties, sprake van fibrinolyse in de buik.
In het plasma van peritonitis patiënten waren in vergelijking met controle patiënten alleen het uPA en de FbDP significant verhoogd.
Er mag worden gesteld dat zowel de stolling als het fibrinolytische systeem in het buikvocht van patiënten met een bacteriële peritonitis is gestimuleerd. Het lijkt er echter op dat de fibrinolytische activiteit onvoldoende is ten gevolge van de gelijktijdige remming van plasminogeen activatoren.

Op zoek naar de oorzaak van adhesie- en abcesvorming in de buik worden in **Hoofdstuk 9** de activiteiten van tPA en PAI gemeten in het buikvocht van ratten met een faecale peritonitis en gerelateerd aan de mate van uitbreiding van de peritoneumbeschadiging.

Hoewel de tPA activiteit gedurende de hele studieperiode van 8 dagen laag was, bleek deze hoger in ratten met peritonitis. De PAI activiteit was significant hoger in ratten met een peritonitis dan in controle ratten. In beide groepen ratten was histologisch gezien de peritoneumbeschadiging hetzelfde. Er bestond geen verband tussen de mate van uitbreiding van de peritoneumbeschadiging en de tPA en PAI activiteit. Waarschijnlijk is de toegenomen PAI activiteit in het buikvocht van ratten met een faecale peritonitis de belangrijkste oorzaak van de afgenomen fibrinolyse in de buikholte. Andere bronnen dan het mesotheel zouden verantwoordelijk kunnen zijn voor deze tPA en PAI activiteit.

Hoofdstuk 10 bevat gegevens over de in vitro interactie tussen humaan recombinant tPA (rtPA) en het fibrinolytische systeem van de rat. Er werden diverse kruisreacties tussen humaan rtPA en rattefactoren gevonden: humaan rtPA lost 'rattestolsels' op, humaan rtPA leidt tot plasmine gemedieerde afbraak van fibrinogeen en fibrine, en humaan rtPA wordt geremd door ratte-PAI. 'Rattestolsels' lijken echter meer resistent te zijn tegen afbraak door humaan rtPA dan humane stolsels. Bovendien is de laboratoriumtest, waarmee fibrine afbraakprodukten worden aangetoond, minder gevoelig voor ratte-FbDP dan humane-FbDP. Streptokinase was niet in staat rattestolsels op te lossen.
Vanwege de interactie tussen humaan rtPA en delen van het fibrinolytische systeem in de rat, is de rat een goed proefdier om beïnvloeding van de fibrinolytische activiteit door middel van humaan rtPA tijdens peritonitis te bestuderen.

In **Hoofdstuk 11** wordt de pharmacokinetiek van intraperitoneaal toegediend humaan rtPA bij ratten met een faecale peritonitis beschreven. Allereerst werd de endogene tPA en PAI activiteit in het buikvocht en het plasma gemeten. Vervolgens werd zowel het tPA antigeen als de tPA activiteit gemeten na éénmalige intraperitoneale toediening van 0.5 mg/ml of 2.0 mg/ml humaan rtPA.
Het bleek dat de endogene tPA activiteit in het buikvocht slechts licht verhoogd was, terwijl de PAI activiteit een sterke toename liet zien bij peritonitis, met een piek op 16 uur na het ontstaan van de peritonitis. In het plasma was alleen de PAI activiteit verhoogd.
Met beide doseringen humaan rtPA werd er gedurende de eerste 24 uur een aanzienlijke verhoging van de fibrinolytische activiteit bereikt in het buikvocht van alle ratten. In

ratten met een peritonitis was de gemeten tPA activiteit echter lager dan te verwachten was op basis van de gemeten tPA antigeen spiegels. Met beide doseringen bleef de plasma tPA activiteit relatief laag. Bij het gebruik van een gel als medium, waarin rtPA werd opgelost in plaats van Ringer's lactaat, was de tPA antigeen concentratie in het plasma lager.
Een éénmalige toediening van 0.5 mg/ml of 2.0 mg/ml humaan rtPA is in staat de fibrinolytische activiteit in de buikholte gedurende 24 uur te verhogen. Het intra-peritoneaal gebruik van humaan rtPA bij peritonitis wordt mogelijk nadelig beïnvloed door de hoge PAI concentraties in het buikvocht. Het risico van bloedingen bij gebruik van rtPA in de buik bij peritonitis is zeer waarschijnlijk laag.

In **Hoofdstuk 12** wordt verslag gedaan van het effect van verhoging van de fibrinolytische activiteit in de buikholte op het ontstaan van abcessen bij ratten met een faecale peritonitis door het intra-abdominaal toedienen van humaan rtPA (beschrijving peritonitis model in Hoofdstuk 9).
Er kwamen minder intra-abdominale abcessen voor bij ratten behandeld met rtPA, opgelost in methyl hydroxy propyl cellulose (MHPC) gel (0.5 mg/ml) dan bij controle ratten, behandeld met MHPC alleen danwel met Ringer's lactaat oplossing. Uit de abcessen werd niet alleen de ingebrachte *E. coli* gekweekt, maar ook bacteriën afkomstig uit de darm van de rat, hetgeen een aanwijzing is voor bacteriële translocatie. De mortaliteit van ratten behandeld met rtPA was hoger dan van controle ratten, hoewel tot onze verbazing een bacteriëmie niet werd aangetoond. Indien de ratten werden blootgesteld aan een hogere dosis *E. coli* in het inoculum (10^6 kolonie vormende eenheden per ml) trad wel in een vroeg stadium een bacteriëmie op. Echter dit kon niet worden gerelateerd aan een toegenomen sterfte. Er was geen relatie tussen het gebruik van rtPA en het optreden van bloedingscomplicaties.
Recombinant tPA voorkomt de vorming van intra-abdominale abscessen in ratten met een faecale peritonitis. De vroeg optredende bacteriëmie en de verhoogde mortaliteit zijn belangrijke nadelen van het intra-abdominaal gebruik van rtPA in dit rattemodel.

In **Hoofdstuk 13** wordt het effect van gentamicine bestudeerd op bacteriëmie en mortaliteit, die worden veroorzaakt door het intra-abdominaal toedienen van rtPA bij ratten met een faecale peritonitis, zoals werd beschreven in het vorige hoofdstuk.
Bij de hoge dosering *E. coli* (10^8 kolonie vormende eenheden per ml) leidde gentamicine

tot een significante verlaging van: (1) het aantal ratten met een bacteriëmie, (2) de bacteriële concentratie in het bloed en (3) de mortaliteit. Gentamicine voorkwam sterfte door rtPA bij de lage dosering *E. coli* (10^4 kolonie vormende eenheden per ml), hoewel geen enkele rat een bacteriëmie ontwikkelde. Dieren die met rtPA waren behandeld hadden significant minder abcessen dan dieren die niet met rtPA waren behandeld. Gentamicine had geen invloed op het aantal abcessen.
In conclusie: gentamicine verlaagt het aantal bacteriëmieën en de mortaliteit bij ratten met een faecale peritonitis, die intraperitoneaal rtPA krijgen toegediend om intra-abdominale abcessen te voorkomen.

Hoofdstuk 14 bevat de algemene beschouwing. Bij een intra-abdominale infectie is zowel de stollingscascade als het fibrinolytische systeem in de buikholte geactiveerd. Het lijkt er echter op dat de fibrinolytische activiteit onvoldoende is gestimuleerd ten gevolge van een gelijktijdige verhoging van remmers in het fibrinolytische systeem. Dientengevolge kan fibrine aanwezig blijven, zich organiseren en vervolgens een bron van restinfectie worden in de buik. In ratten met een faecale peritonitis kan restinfectie op een veilige manier worden voorkomen door het verhogen van de fibrinolytische activiteit in de buikholte met behulp van rtPA, in combinatie met antibiotica.
De veranderingen in de tijd van het stollings- en fibrinolytische proces in de buikholte tijdens peritonitis en na het verwijderen van de bron van infectie moeten nader worden bestudeerd. De vraag waarom in sommige omstandigheden fibrine in de buikholte aanwezig blijft en leidt tot restinfectie, en er een indicatie bestaat voor fibrinolytische therapie, kan hiermee mogelijk worden beantwoord.

LEKENPRAATJE

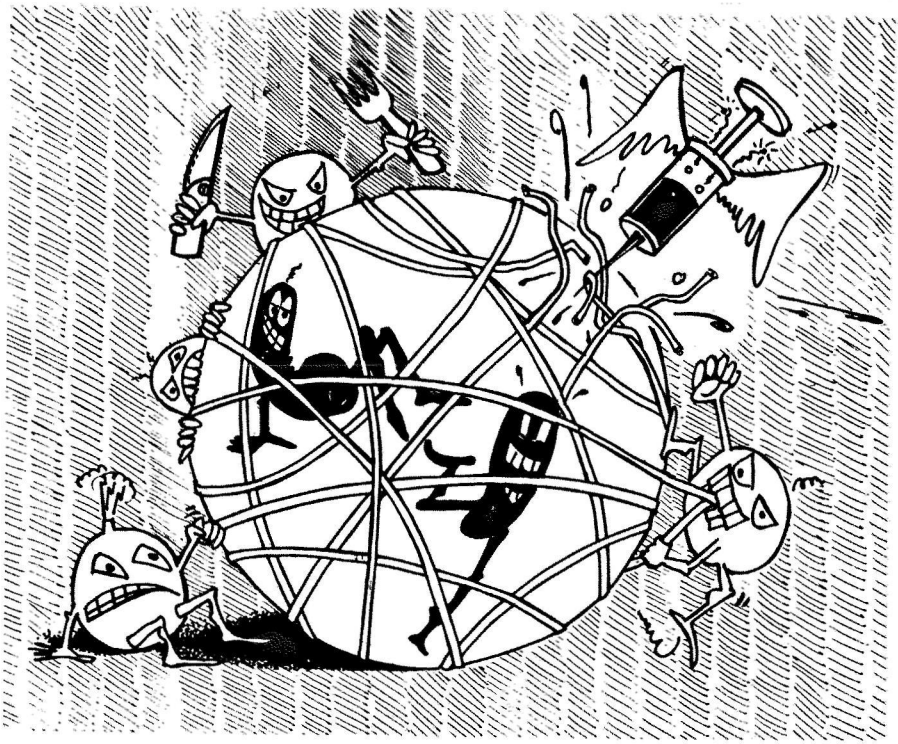
voor leken die leken lijken
en niet-leken die leken blijken

In dit proefschrift heeft fibrine een centrale rol. Fibrine is de 'weefsellijmstof' in ons lichaam. Het is het eindproduct van het stollingsproces. Dit proces komt bijvoorbeeld op gang wanneer er een wond ontstaat of wordt gemaakt met letsel van bloedvaten. Een netwerk van fibrine dicht dan het gat in een bloedvat en zo komt de bloeding tot staan. Deze werking van fibrine is in principe tijdelijk. Na enige tijd wordt fibrine weer afgebroken en treedt definitieve wondgenezing op.
Ook bij ontstekingen in het lichaam wordt fibrine gevormd. Zo wordt tijdens een operatie van patiënten met een blindedarmontsteking of een doorgebroken maagzweer meestal fibrine in de buik waargenomen. Deze fibrine vormt netwerken (verklevingen) en omsluit hiermee bacteriën en andere schadelijke stoffen die in de buik zijn gekomen. Zo kunnen deze niet in de bloedbaan komen en aanleiding geven tot een bloedvergiftiging. Dit is dus van groot voordeel voor de patiënt.
Een nadeel is echter dat bacteriën worden afgeschermd van afweercellen van het eigen lichaam, die normaal gesproken bacteriën doden en afbreken. Ook werken antibiotica veel minder goed tegen bacteriën die in fibrine zijn 'gevangen'. Hierdoor zijn bacteriën in staat, in een min of meer beschermde omgeving van fibrine, zich te vermenigvuldigen en allerlei stoffen af te geven die schade toebrengen aan het lichaam. Op den duur ontstaat een abces in de buik. Patiënten hebben dan hoge koorts, last van zweten, buikpijn, verminderde eetlust en vermagering. Uiteindelijk kunnen ze zelfs overlijden aan een dergelijk abces.
Dus: fibrine heeft zowel een gunstige werking als een ongunstige werking bij een infectie in de buik.

De gunstige werking van fibrine -het voorkomen dat bacteriën via de bloedbaan schade aanrichten- kan tegenwoordig goed worden overgenomen door antibiotica. Deze zorgen ervoor dat de bacteriën die in de bloedbaan komen onmiddellijk worden gedood. Tegen de ongunstige werking van fibrine -het ontstaan van abcessen- is tot op heden nog geen afdoend middel gevonden.

Er bevinden zich in de buik stoffen, afkomstig van cellen die de vrije buikholte bekleden, die fibrine kunnen oplossen (=tPA). Wij hebben aangetoond dat tPA in verhoogde hoeveelheden aanwezig is in de buikholte bij een buikvliesontsteking. Toch worden

de fibrine-netwerken niet opgelost; enerzijds omdat er een continue aanmaak is van fibrine, anderzijds omdat de tPA wordt geremd. Tijdens een infectie bleken er namelijk ook grote hoeveelheden remmende stoffen van tPA (=PAI) aanwezig te zijn in de buikholte. Er is dus een verstoring ontstaan in de balans tussen fibrine-aanmaak en -afbraak op een zodanige wijze dat deze fibrine-netwerken langdurig in de buik aanwezig blijven en zich zelfs verstevigen.



'Breakthrough' by Marijke van Balen

Dat fibrine de bron is van abcessen in de buikholte bij infecties, is al enige tijd geleden onderkend. Om deze reden zijn operatiemethoden ontwikkeld, die gericht zijn op het steeds maar weer verwijderen van fibrine-netwerken die verklevingen vormen tussen de diverse buikorganen. Deze methoden hebben als nadeel dat vaker dan één keer moet worden geopereerd en dat elke nieuwe operatie weer leidt tot nieuwe fibrinevorming. Ook kan het verwijderen van verklevingen met de hand, de schaar of het pincet gaatjes veroorzaken in de darm of bloedvaten, waardoor ontlasting in de vrije buikholte kan

komen of bloedingen optreden en hierdoor de infectie wordt verergerd. Door deze complicaties hebben dergelijke operatiemethoden in het algemeen niet het gewenste resultaat opgeleverd.

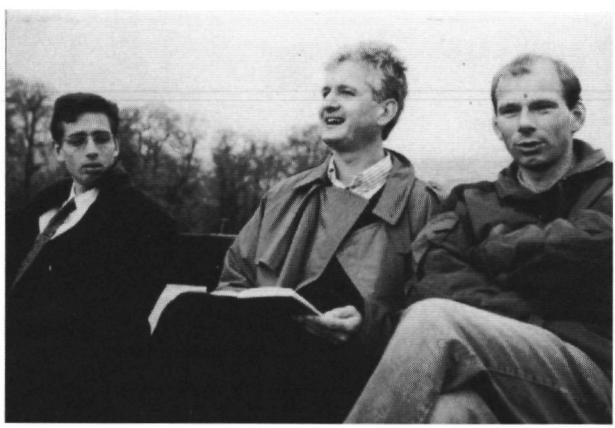
Een andere mogelijkheid om fibrine kwijt te raken is de locale verhoging van fibrine-oplossende stoffen. Dit kan door bijvoorbeeld een dergelijke stof in de buik toe te dienen. Wij hebben de werking van een dergelijke fibrine-oplosser (=tPA) getest in ratten met een buikvliesontsteking. Het bleek inderdaad dat bij ratten die werden behandeld met tPA minder abcessen in de buikholte aanwezig waren.
Naar verwachting nam wel het aantal bacteriën in de bloedbaan toe bij het gebruik van tPA. Dit ging ook gepaard met een verhoogde sterfte bij deze ratten. Echter zowel de hoeveelheid bacteriën in de bloedbaan alsmede de sterfte kon worden verminderd door het gelijktijdig gebruik van antibiotica. Waarschijnlijk krijgen bij gebruik van tPA de natuurlijke afweercellen (en antibiotica) de kans om de bacteriën in de vrije buikholte te doden. De combinatie van tPA en antibiotica lijkt dan ook doeltreffend in de strijd tegen het ontstaan van abcessen na een ernstige buikvliesontsteking.

Natuurlijk moet een dergelijk fibrine-oplosmiddel eerst nog worden getest bij grotere proefdieren en bij mensen om uiteindelijk een adequaat middel in handen te hebben om abcessen na een buikvliesontsteking te kunnen voorkomen.
Er is nog een lange weg van onderzoek te gaan...

NAWOORD

Doorgewinterde proefschriftlezers beginnen met het na- of dankwoord. Om deze reden is het nawoord vooral een voorwoord geworden. Bij dezen...

Het idee achter dit proefschrift is ontstaan in Wenen op een bankje voor paleis Schönbrunn (zie foto). tPA moest één van de ingrediënten worden van de 'ideale' spoelvloeistof voor 'vieze' buiken. Vol enthousiasme het concept uitgewerkt, onder genot van spinazie met knoflook (kok: Rob B.) en witte wijn, gevolgd door de tennismatch van het jaar. Na ruim vijf jaar het resultaat: geen 'ideale' spoelvloeistof, tPA werkt, maar hoe precies? Een nieuw Weens avontuur is wellicht noodzakelijk voor de 'juiste oplossing'.
Enfin, beste Rob Bleichrodt, die grijze haren had ík je kunnen bezorgen. Je bezigde meermalen de term 'zelfrijzend bakmeel', maar vaak ontbrak het gist. Je bent meer dan de ideeënbedenker, de initiator en begeleider van dit proefschrift: een goede vriend met wie vele gezellige uurtjes zijn doorgebracht, waarbij er (onder andere) over het vak werd gepraat. Rob, zeer bedankt, je weet wel...



Co-promotor dr. J. van der Meer, beste Jan, het is goed zaken doen met jou. Stagnatie in het onderzoek werd niet getolereerd. Je fibrinolytisch vermogen is ongekend, evenals je enthousiasme, efficiëntie, kennis van zaken en no-nonsense techniek van communiceren. Hartelijk dank!

Promotor prof.dr. R.J.A. Goris, beste Jan, van mijn overstap van Groningen naar Nijmegen heb ik geen spijt. Er is een goede verstandhouding ontstaan. Dank voor de begeleiding bij het laatste deel van het proefschrift. De tweede set begint, zet je schrap!

Dank ben ik verschuldigd aan dr. V.J.J. Bom. Beste Victor, jij was de bewaker van de assays en legde steeds genadeloos de zwakke plek in onze wetenschappelijke ideeën bloot. Discussiëren met jou was niet altijd even makkelijk, maar ik heb je bijdrage zeer gewaardeerd.

Dr. W.J. Sluiter, beste Wim, sinds je de mathematische modellen hebt gemaakt van het glucose-metabolisme bij portaal versus systemisch geschakelde pancreatica, ben ik niet meer van je losgekomen. Van het berekenen van CIR-waarden op een veredelde telmachine tot ingewikkelde statistische bewerkingen op een 486-er, je bent onnavolgbaar. Zonder jou had dit werk niet het predikaat 'statistisch relevant' gekregen. Wim....chapeau!

Members of the manuscript committee, prof.dr. H. Wacha, prof.dr. H.M. Vemer and prof.dr. J.A.A. Hoogkamp-Korstanje, thank you for the speed in reviewing this manuscript during the warm summer of 1995.

Een artikelenbevattend proefschrift heeft mede-auteurs. Een ieder heeft, op zijn of haar eigen terrein, díe bijdrage geleverd die nodig was om 'submitted' om te zetten in 'accepted'. Bedankt!

Gedurende enkele jaren heb ik het stollingslaboratorium en de dependance van het streeklab onveilig gemaakt met buisjes bloed, buikvocht en pus. De flexibiliteit, waarmee medisch volk van allerlei pluimage tegemoet werd getreden, was opvallend. Haast ongewild ging ik me als chirurg thuisvoelen tussen agarplaten, erlenmeyers, **-80**-ers en ijklijnen. Dames en heren analisten en secretaresses, dit was een verdienste van jullie allemaal, houden zo!

Gedurende de laatste anderhalf jaar heeft Myrna (Miranda) Verstegen zich gebogen over de wanordelijke papiermassa bestaande uit manuscripten, inleidingen en lijsten met referenties. Dichterlijke vrijheden werden kordaat ingedamd. Ze heeft kleur gegeven aan dit boekje, zonder kleurbepalend te zijn geweest. Lieve Myrna, je bent voortreffelijk.

Mijn ouders dank ik voor de mij geboden mogelijkheden tijdens mijn jeugd thuis en tijdens mijn studieperiode. Vele dierbare herinneringen zijn verbonden aan het ouderlijk huis, hetgeen 'thuiskomen' nog steeds tot een aangename bezigheid maakt.

Marianne, van jou mocht ik geen hoogdravende woorden schrijven over 'ontberingen', 'gebrek aan gezinsleven', 'de verloren uurtjes inhalen', of 'dankzij' danwel 'ondanks'.

Daarom houd ik het kort: ik hou van je.

Tenslotte dank ik alle onderstaande personen die in de afgelopen jaren in meer of mindere mate betrokken zijn geweest bij dit proefschrift (in wording). Indien ik iemand heb vergeten...volgende keer beter!

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CURRICULUM VITAE

Harm van Goor

1957	Geboren te Zwolle
1975	Eindexamen Gymnasium ß, Carolus Clusius College te Zwolle
1983	Artsexamen Rijksuniversiteit te Groningen
1983	AGNIO Afd. Heelkunde, Academisch Ziekenhuis Groningen
1984-1986	Transplantatiecoördinator, Academisch Ziekenhuis Groningen
1986-1989	Opleiding Algemene Chirurgie, Sophia Ziekenhuis te Zwolle (opleider: Dr. W. van Rooyen)
1989-1992	Opleiding Algemene Chirurgie, Academisch Ziekenhuis Groningen (opleider: Prof.dr. R. van Schilfgaarde)
1992-1994	'Junior-chirurg', Academisch Ziekenhuis Groningen (aandachtsgebied Vaatchirurgie)
1994-heden	Chirurg Academisch Ziekenhuis Nijmegen St. Radboud (aandachtsgebied Gastro-Intestinale Chirurgie)

Harm van Goor is getrouwd met Marianne Haarhuis. Samen zijn zij ouders van Mark, Harriëtte en Ellen.

STELLINGEN

behorend bij het proefschrift

FIBRINOLYTIC THERAPY IN GENERALIZED PERITONITIS TO PREVENT INTRAABDOMINAL ABSCESS FORMATION
an experimental and clinical study

Harm van Goor

2 februari 1996

1. De pathofysiologie van het ontstaan en 'oplossen' van adhesies is nog (lang) niet opgehelderd.

2. De fibrinolytische activiteit in de buikholte berust niet op een aan-uit fenomeen, maar op een nauwkeurige balans tussen remmers en stimulatoren van de fibrinolyse.

3. Hoge PAI spiegels in het plasma tijdens peritonitis maken toepassing van hoge doseringen rtPA in de buik mogelijk, zonder dat systemische complicaties zullen optreden.

4. Meer dan vier geplande relaparotomieën bij 'septische' buiken biedt geen voordelen voor de patiënt.

5. Na een acute necrotiserende pancreatitis is ook op de lange termijn een normale glucosetolerantie en een normale insuline-gevoeligheid niet te verwachten.

6. Het 'systemisch schakelen' van een pancreastransplantaat is onlogisch.

7. Zolang er een tekort bestaat aan orgaandonoren is er geen indicatie voor levertransplantatie bij patiënten met maligne tumoren van de lever of de galwegen.

8. Congenital hypoplasia of the scaphoid bone must be regarded as a radial longitudinal hypoplasia, in which the limb is affected in a proximal-distal sequence, and not as an intercalary defect *(J Hand Surg 1989;14A:291-4)*.

9. NS 1996: eerste klas staat beter!

10. Na een primaire traumatische schouderluxatie bij jonge mensen moet diagnostiek worden verricht naar het voorkomen van een afscheuring van het labrum glenoïdale en indien aanwezig moet dit arthroscopisch worden gefixeerd.

11. Gezien het stedelijk karakter van de recente (burger)oorlogen en de toenemende politieke bereidheid tot inzet van Nederlandse militairen daarbij, is het aan te bevelen in plaats van de mooiste natuurgebieden oude vervallen stadswijken te gebruiken als militair oefenterrein.

12. Een goede transplantatiecoördinator is bij-de-hand.

13. Het heffen van mannenbelasting onder het motto 'de vervuiler betaalt' geeft een nieuwe dimensie aan het begrip 'foute mannen'.

14. In interviews met publieke media overschat de medicus vaak de kennis van het publiek, maar onderschat diens gezond verstand.

15. Als in de operatiekamer de sfeer om te snijden is, is er geen sfeer om te snijden.

16. Hoewel veel beproefd is de Nederlandse kookkunst een smakeloze vorm van kunst.

17. Een echte omloop is nooit weg.