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The Use of Animal Models to Study Bacterial Translocation During Acute Pancreatitis

L. P. van Minnen · M. Blom · H. M. Timmerman · M. R. Visser · H. G. Gooszen · L. M. A. Akkermans

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Abstract Infection of pancreatic necrosis with intestinal flora is accepted to be a main predictor of outcome during severe acute pancreatitis. Bacterial translocation is the process whereby luminal bacteria migrate to extraintestinal sites. Animal models were proven indispensable in detecting three major aspects of bacterial translocation: small bowel bacterial overgrowth, mucosal barrier failure, and disturbed immune responses. Despite the progress made in the knowledge of bacterial translocation, the exact mechanism, origin and route of bacteria, and the optimal prophylactic and treatment strategies remain unclear. Methodological restrictions of animal models are likely to be the cause of this uncertainty. A literature review of animal models used to study bacterial translocation during acute pancreatitis demonstrates that many experimental techniques per se interfere with intestinal flora, mucosal barrier function, or immune response. Interference with these major aspects of bacterial translocation complicates interpretation of study results. This paper addresses these and other issues of animal models most frequently used to study bacterial translocation during acute pancreatitis.

L. P. van Minnen
e-mail: 1pvminnen@gmail.com

M. Blom
e-mail: drs.m.blom@gmail.com

H. M. Timmerman
e-mail: h.timmerman@chir.azu.nl

H. G. Gooszen
e-mail: h.gooszen@umcutrecht.nl

L. P. van Minnen · M. Blom · H. M. Timmerman · H. G. Gooszen · L. M. A. Akkermans (✉)
Department of Surgery, Gastrointestinal Research Unit,
University Medical Center Utrecht,
P.O. Box 85500, Utrecht 3508 GA, The Netherlands
e-mail: L.M.A.Akkermans@umcutrecht.nl

L. P. van Minnen · M. Blom · H. M. Timmerman · M. R. Visser · H. G. Gooszen · L. M. A. Akkermans
Department of Medical Microbiology,
University Medical Center Utrecht,
Utrecht, The Netherlands

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Introduction

Experimental models of acute pancreatitis exist for almost 150 years, with Claude Bernard first describing experimental pancreatitis by injection of bile and olive oil into the pancreatic duct of a rabbit.[1] Ever since, animal experiments were indispensable in providing insight in pathophysiology and treatment of acute pancreatitis. Experimental studies have major advantages over clinical studies, such as the availability of study subjects, standardization of disease severity, ability to perform invasive tests, extensive tissue sampling, and the possibility to test prophylactic treatment strategies.[2] Despite these advantages, some major aspects of the pathophysiology of acute pancreatitis remain unclear, mortality in severe acute pancreatitis is still as high as 5–28%, and optimal treatment strategies remain a topic of debate.[3,4]

In 1986, Beger et al. demonstrated a link between the intestinal flora, infection of pancreatic necrosis, and clinical outcome in patients with severe acute pancreatitis.[5] At the present time, infection of pancreatic necrosis is still regarded to be a main predictor of outcome during severe acute pancreatitis, and bacterial translocation of intestinal flora is considered to be the cause.[4]

Changes in intestinal motility and the associated shift of intestinal flora, mucosal barrier function, and the immune system were identified as pivotal aspects of bacterial translocation during acute pancreatitis.[6–11] This has greatly increased the understanding of bacterial translocation, but better insight into the exact mechanism of bacterial translocation and subsequent infection of pancreatic necrosis is needed to develop adequate prophylaxis and treatment strategies for patients with severe acute pancreatitis.

A multitude of animal models were used to study the mechanism of bacterial translocation, including radiolabeling, plasmid-labeled bacteria, or fluorescent beads.[12–15] Despite all these efforts, however, the exact origin, route, and mechanism of bacterial translocation causing infection of pancreatic necrosis are still unclear. The main reason for this uncertainty is the lack of an "ideal" animal model of acute pancreatitis to study pathophysiology of bacterial translocation and its treatment. The ideal model should be minimally invasive, standardized, reproducible, and resemble etiology, pathophysiology, disease course, and outcome of clinical acute pancreatitis, including response to treatment.[2] Experimental models used to study bacterial translocation in acute pancreatitis and its treatment all seem to have methodological restrictions that complicate the interpretation of study results. In 2000, Foitzik et al. reviewed the use of animal models of acute pancreatitis and their suitability for evaluating therapy and concluded that animal models should be designed to mimic etiology and clinical course of human pancreatitis to increase their value.[2] In addition, we would like to discuss the value animals studies and experimental models of acute pancreatitis have in face of their interference with one or more of the known aspects of bacterial translocation: intestinal motility and flora, mucosal barrier function, or the immune system.

The aim of this paper is to provide useful insights into the use of animal models to study bacterial translocation during acute pancreatitis, in the light of current knowledge of pathophysiology.

Animal Species and Housing Conditions

Before the late 1970s, larger laboratory animals such as dogs were predominantly used to study acute pancreatitis. But since the introduction of models of acute pancreatitis in small laboratory animals, mice or rats are generally favored for financial and ethical or practical reasons. Because of physiological and anatomical differences between species, choice of laboratory animal has important implications on the study results and extrapolation to the human situation.

Intestinal flora differs between animal species, largely depending on dietary demands and anatomical differences of the gastrointestinal tract and habits.[16–18] The protein-rich diet of dogs or cats results in lower counts of endogenous lactobacilli and higher counts of potential pathogens (e.g., clostridia species), compared to rats or mice with fiber-rich diets. Coprophagy, demonstrated by most rodents, also influences intestinal flora, resulting in higher counts of gram-negative bacteria in the proximal gastrointestinal tract.[19,20] Also, rats and mice are often bred and kept under specific pathogen-free conditions, introducing modifications of intestinal flora.

Intestinal barrier function also differs between species. In an experiment comparing small intestinal permeability between humans and rats, significant interspecies variation in urinary recovery of orally delivered mannitol was observed.[21]

Anatomical differences between species should also be considered. The relative size of the jejunum, ileum, cecum, and colon of different laboratory animals can influence origin and route of bacterial translocation during acute pancreatitis. In humans, retroperitoneal connections between the intestines and pancreas can greatly affect the clinical course of the disease.[22] Similar to humans, the dog pancreas is situated retroperitoneally. Rat and mouse pancreata, however, are almost fully enveloped by peritoneum, resembling a more intraperitoneal localization. Variation in retroperitoneal connections between intestines and the pancreas offers different routes for bacteria to translocate without being exposed to intraperitoneal immune cells.[23]

Experiments using small animals (e.g., mouse or rat) usually incorporate a larger number of animals compared to experiments with large laboratory animals (e.g., cat or dog). The use of a larger number of small laboratory animals improves statistical power of an experiment. On the other hand, the use of larger animals could resemble human pathophysiology better, but a smaller number of animals means lower statistical power and increased potential false negative or false positive results.

Models of Acute Pancreatitis

An abundance of animal models of acute pancreatitis is used to investigate bacterial translocation. Only models most frequently used for this purpose will be discussed. Baseline characteristics of the discussed models and their potential effects on intestinal flora, mucosal barrier, and immune function are summarized in Tables 1 and 2.

Table 1 Characteristics of Several Animal Models of Acute Pancreatitis

Model	Animal Species	Pancreatic Necrosis	Pancreatic Infection	Mortality	Invasiveness
Duodenal loop[24,25]	Rat	No	Considerable	High	Laparotomy
Choline-deficient diet[30–32]	Mouse	Yes	Little	High	Minimal
Duct ligation[34–37]	Rat/opossum	No/Yes	Little	Low	Laparotomy
Cerulein[44]	Mouse/rat	Yes/No	Little	Low	Minimal
Duct perfusion[48]	Rat/dog/pig	Yes	Considerable	Moderate to high	Laparotomy
Duct perfusion + cerulein[52]	Rat	Yes	Considerable	Moderate	Laparotomy

Duodenal Loop

Closing the duodenal lumen proximally and distally to the papilla of Vater results in reflux of the duodenal contents enclosed in the loop, including bile and pancreatic secretions, into the biliopancreatic duct.[24] In rats, this leads to acute pancreatitis of varying severity.[25] Discontinuation of the gastrointestinal tract leads to mucosal atrophy and functional changes to the mucosal barrier.[26] Furthermore, obstruction of bile flow into the intestine was shown to reduce intestinal motility, causing small bowel bacterial overgrowth and increased bacterial translocation.[27–29] Another major downside is the occurrence of reflux of duodenal contents, including bacteria, into the biliopancreatic duct. These obvious drawbacks of this model in experiments concerning bacterial translocation are the cause of its limited popularity.

Ethionine-supplemented Choline Deficiency

Lombardi et al.[30] described severe acute pancreatitis in young female mice after feeding a choline-deficient, ethionine-supplemented (CDE) diet.[31] Acute hemorrhagic pancreatitis ensues, as well as diffuse intraperitoneal fat necrosis and several systemic effects such as acidosis, hypoxia, and hypovolemia. In this model, mortality ranges from 0 to 100% after 4 days and can be controlled by varying the duration of the choline-deficient diet.[32] To ensure homogeneity and reproducibility, sex, age, and weight of the mice have to be closely matched, as well as food intake of all animals.[32]

Apart from these practical downsides of the model, systemic complications unrelated to pancreatitis (e.g., parotitis and fatty liver disease) render the model less useful for investigating systemic events (e.g., immune response) of acute pancreatitis.[31] Little is known of the effect of ethionine suppletion or choline deficiency on intestinal flora or mucosal barrier function. But the most important drawback of this model to study bacterial translocation is the low incidence of pancreatic infection (3–8%), even in severe necrotizing pancreatitis.[33]

Biliopancreatic Duct Ligation

In the duct ligation model, the common biliopancreatic duct is surgically clipped or tied at the sphincter of Oddi

Table 2 Aspects of Bacterial Translocation and Potential Confounding Factors of Animal Models

Aspect	Confounding Factor	Model
Intestinal motility and flora	Animal species	Potentially all models
	Housing conditions (SPF)	Potentially all models
	Diet	CDE diet
	Analgesics	Invasive models
	Laparotomy	Invasive models
	Bile flow	Duct ligation
	Cerulein	Cerulein models
	Intestinal manipulation	Invasive models
Mucosal barrier function	Stress	Potentially all models
	Diet	CDE diet
	Anesthetics	Invasive models
	Pancreatic proteases	Duct ligation
	Intestinal manipulation/puncture	Duct perfusion
Immune system	Stress	Potentially all models
	Diet	CDE diet
	Disease course/severity	Species-dependent
	Obstructive jaundice	Duct ligation, duodenal loop
	Intestinal manipulation	Invasive models

complex. The resulting obstruction of pancreatic secretions and potential biliary reflux into the pancreatic duct produce moderate pancreatitis, characterized by edema, moderate inflammation and hemorrhage, fat necrosis, and minimal acinar cell necrosis. Only in the American opossum does biliopancreatic duct ligation leads to severe acute pancreatitis with considerable necrosis.[34–37]

This model of acute pancreatitis greatly interferes with the pathophysiology of bacterial translocation. Obstruction of bile flow into the intestine causes small bowel bacterial overgrowth and bacterial translocation.[28] Also, exclusion of pancreatic proteases in the gut lumen alters intestinal permeability.[38,39] Apart from effects on the intestinal flora and mucosal barrier function, obstruction-induced jaundice also causes impairment of the immunesystem.[40–42] These effects complicate the interpretation of bacteriological results to study bacterial translocation.

Cerulein Infusion

Infusion of low doses of cerulein, a cholecystokinin analog, enhances production of pancreatic exocrine cell secretions without cell necrosis. In most species, infusion of supramaximal doses results in a decrease of secretion and acute pancreatitis with interstitial edema and inflammatory cell infiltration.[43] In mice, cerulein causes severe acute pancreatitis with necrosis of 40% of acinar cells.[44] In rats and other animals, however, cerulein-induced pancreatitis is usually mild and generally self-limiting. Moreover, pigs are reported to be insensitive to cerulein hyperstimulation.[45] It should be noted that cerulein is known to affect intestinal motility. Studies investigating the use of cerulein in man have shown absence of recognizable migrating motor complexes with decreased colonic transit time.[46] In general, experimental acute pancreatitis is associated with reduced small bowel motility, resulting in small bowel bacterial overgrowth and increased bacterial translocation to extraintestinal sites.[6,47] Thus, cerulein may interfere with intestinal flora by altering intestinal motility. Investigators should keep this in mind when designing a study and interpreting study results.

Biliopancreatic Duct Perfusion

Duct perfusion models are currently the most popular models of acute pancreatitis. Induction of acute pancreatitis involves infusion of bile, bile salts with or without bacteria, or activated pancreatic enzymes into the (bilio-)pancreatic duct. Early experiments mainly involved dogs, but currently, rats are used most frequently. Severity and reproducibility of acute pancreatitis and ensuing bacteriological results strongly depend on infusate, infusion pressure, volume, and time.[48]

The most commonly used infusates are solutions containing various concentrations of bile salts of varying hydrophobicity. Both chemical and pressure effects of infusion were suggested to play a major role in the pathogenesis of pancreatitis in perfusion models.[48,49] In both chemical- and pressure-induced pancreatitis, destruction of the pancreatic duct mucosal barrier is the key event. This is followed by pancreatic edema, autolysis, reduction of pancreatic blood flow, and, in severe cases, destruction of pancreatic parenchyma and formation of pancreatic necrosis.[50] Uncontrolled pressure-related damage causes variation in severity of the induced acute pancreatitis between study subjects, and thus should be avoided. Several experiments were performed to assess maximal pancreatic duct pressure before rupture of the duct epithelium causing increased and uncontrolled severity of acute pancreatitis. Data are conflicting, with rupture pressures varying from 15 to 82 mmHg.[48,49,51,52] A maximum infusion pressure of 30 to 50 mmHg is currently accepted for rat models.

Perfusion is usually performed by puncturing the duodenum and cannulating the papilla of Vater. The introduction of duodenal bacteria, through the papilla of Vater into the biliopancreatic duct could potentially be a confounding factor in transduodenal duct perfusion models. It was demonstrated, however, that significant bacterial infection of the pancreas ($>1\times10^2$ colony forming units per gram) because of the surgical procedure does not occur.[53]

Advantages of this model are the quick procedure of acute pancreatitis induction and the reproducibility of results. Other than duodenal puncturing and intestinal handling during surgery, both potentially affecting mucosal barrier function, no direct effects on intestinal flora or immune function are expected in this model.

Biliopancreatic Duct Injection and Cerulein Hyperstimulation

The combination of retrograde infusion of bile salts with superimposed cerulein hyperstimulation in rats was introduced by Schmidt et al. and was advocated as "a better model for evaluating therapy."[52] Although the disadvantages described for biliopancreatic duct injection and cerulein hyperstimulation all apply to this model, it was proven a very valuable model to examine bacterial translocation and treatment strategies. The major advantages are that histological and qualitative bacteriological results as well as reaction to treatment and disease course resemble human acute pancreatitis more closely than other models.[2,52] Although proven a very valuable model, potential model-related confounding factors as described above should always be kept in mind when interpreting results.

Disease Course

Especially in the severe form of acute pancreatitis, systemic events can be divided into two phases: early proinflammatory and late immunosuppressive.[54] In severe acute pancreatitis, the early phase is associated with a systemic inflammatory response syndrome (SIRS), potentially leading to multiple organ failure and early mortality. The late phase is characterized by immunosuppression, providing opportunity for infectious complications (e.g., infection of pancreatic necrosis) associated with sepsis and late mortality.[2,55] Laboratory animal species and experimental models, however, each show their own disease course of acute pancreatitis.

Animal models were mainly used to investigate the early phase of acute pancreatitis.[56] However, the model described by Schmidt et al. seems the most appropriate to investigate early and late systemic complications, considering that both phases can be discerned.[52,57] In this model, infection of pancreatic necrosis progresses at least until 96 h. When taking into account that disease course is more rapid in small rodents, timing could well correlate with data on the course of severe acute pancreatitis in humans, as described by Foitzik et al.[2], Beger et al.,[4] and Lankisch et al.[58]

Severity

Pancreatic necrosis is produced in several animal models of acute pancreatitis (Table 1). On the other hand, only duct perfusion, with or without superimposed cerulein hyperstimulation, and murine CDE models demonstrate mortality comparable to human necrotizing acute pancreatitis.[32,52,59] Models with high early mortality may be useful to investigate early phase systemic inflammatory response and organ failure, but are less adequate to investigate late infectious complications and associated (multiple) organ failure.

In most models, necrosis needs to be present for pancreatic infection to occur. It needs to be noted that this does not apply for the duodenal loop model in which reflux of duodenal contents into the biliopancreatic duct occurs.[60] In contrast, the murine CDE model produces elaborate necrosis, but is associated with very low rates of pancreatic infection.[33]

Culturing, Controls, and Route of Bacterial Translocation

In all animal models, factors such as analgesia, anesthesia, or surgical techniques can influence bacteriological results. Morphine-like analgesics have a significant effect on bowel motility and cause bacterial overgrowth and translocation to extraintestinal sites.[61] The anesthetic pentobarbital was suspected to be a factor in promoting bacterial translocation in a model of hemorrhagic shock.[62]

Also, stress causes mucosal barrier failure and bacterial translocation.[63] Surgical procedures are stressful events, but animal transport or handling alone could potentially cause stress-induced bacterial translocation. The influence of stress on adrenaline and corticosteroid levels could have its own effect on the function of the immune system, potentially influencing the systemic reaction to acute pancreatitis and bacterial translocation.

Proper sterile surgical techniques are very important when investigating bacterial translocation. If abdominal surgery is involved, control cultures of the peritoneal cavity to trace surgical contamination are of special importance. If peritoneal cultures are found to be positive, extra caution should be taken with interpretation of bacteriological analysis of abdominal organs. In case of surgical contamination or transperitoneal bacterial translocation, the peritoneal covering of the organ samples might be the cause of positive organ cultures, not the bacterial colonization in the organ itself (false positive culture).

Puncturing the duodenum in duct infusion models hypothetically causes spillage of duodenal contents onto the peritoneum, covering all abdominal organs. In rats, however, duodenal contents usually have low bacterial counts, mainly consisting of nonpathogenic lactobacilli only. On the other hand, a duct infusion study by Cicalese et al. reported positive peritoneal cultures at time of induction of pancreatitis of 16.6 to 33.3% of the studied rats.[15] Literature review of different animal models fairly frequently shows positive peritoneal cultures at the time of termination and organ sample collection of rats with acute pancreatitis. Positive peritoneal cultures are observed varying from 0–10% in minimally invasive models of acute pancreatitis (cerulein injection, CDE diet) to 8–100% in more invasive models (duct perfusion with or without cerulein hyperstimulation).[6,14,15,64–66]

Discussion

Changes in intestinal motility and flora, mucosal barrier function, and immune response were established as pivotal aspects in the process of bacterial translocation during acute pancreatitis. Early after the onset of acute pancreatitis, neurohormonal effects result in reduced small bowel motility.[6] This causes stasis of luminal contents and small bowel bacterial overgrowth with potential pathogens, including *Escherichia coli* and *Enterococcus* species. The abundant presence of luminal pathogens forms a challenge for the mucosal barrier. Furthermore, pancreatitis-associated

reduced intestinal blood flow results in mucosal ischemia and reperfusion damage.[67–69] Luminal bacteria, normally held at bay by the mucosal barrier, now have opportunity to penetrate into the intestinal epithelium. Local intestinal inflammation follows, further compromising mucosal barrier function. Pancreatitis and ensuing intestinal inflammation both contribute to a systemic proinflammatory response (SIRS), with damaging effects on distant organs.[70,71] If the systemic response is severe, multiple organ dysfunction syndrome (MODS) might follow.[72,73] If the patient survives the early phase, counterregulatory immunological pathways releasing anti-inflammatory cytokines result in a refractory state characterized by immunosuppression.[74,75] Persistent immunosuppression will render the patient liable for infection of pancreatic necrosis. Multiple organ dysfunction syndrome caused by infectious complications is considered accountable for so-called late mortality or "late septic death."[74,76]

Although animal models were proven indispensable in acute pancreatitis research, model-related problems are most likely the reason for important questions on pathophysiology and treatment strategies to remain unanswered. Current topics of debate include the route and origin of bacterial translocation and optimal prophylaxis and treatment strategies.

Several different routes of bacterial translocation were described and have directed efforts for many prophylactic and therapeutic strategies. Webster et al. showed bacteremia to occur early after induction of acute pancreatitis in CDE-induced acute pancreatitis, suggesting a hematogenous route.[77] Likewise, rapid passage of bacteria into the blood was found in other models of acute pancreatitis.[78] On the other hand, Runkel et al. found bacteria migrating to lymph nodes before their translocation to distant sites in a duct ligation model, suggesting a lymphogenous route.[79] Widdison et al. suggested transperitoneal translocation of bacteria originating from the colon in a feline model of severe necrotizing pancreatitis.[80] Other study groups, including our own, have provided proof of the role of the small bowel in the pathophysiology of bacterial translocation in acute pancreatitis or after morphine administration.[6,61,81]

The model of duct perfusion and cerulein hyperstimulation described by Schmidt et al. was proven very useful because it resembles human disease quite well, considering its biphasic disease course, pancreatic histology, "moderate" mortality, and the bacterial spectrum in pancreatic necrosis.[52] However, whether a confounder is introduced by puncturing the duodenum and cannulating the biliopancreatic duct is unknown. Therefore, to ensure quality of the presented study results, control cultures of the peritoneal cavity should be done when organ samples are analyzed bacteriologically. Peritoneal bacteria can potentially affect bacteriological analysis of all abdominal tissues. Widdison et al. washed abdominal samples before analysis, but this is not commonly performed.[80] A pilot study by Arendt et al. showed that washing removed 94–97% of intraperitoneally injected bacteria.[23] Immunohistologically localizing bacteria can help clarify if positive cultures of abdominal tissues are because of peritoneally located bacteria or actual bacterial colonization in the underlying organ tissue.

When experimentally evaluating therapy, treatment often starts before induction of acute pancreatitis. Obviously, this is an important reason why results cannot directly be translated to the clinical situation. On the other hand, these experimental studies provide proof of principle concerning the tested therapy. If prophylactically successful, the tested treatment strategy might be beneficial when started after the onset of acute pancreatitis and should therefore be further investigated. On the other hand, the faster course of acute pancreatitis in rodent models provides only a very short treatment window between the onset of the disease and early or late phase complications. This may lead to false negative effects of the therapy tested.

In conclusion, animal models of acute pancreatitis are indispensable tools, but model-related drawbacks often interfere with one or more pathophysiological aspects of bacterial translocation, complicating interpretation of results. When the ideal model of acute pancreatitis is not at hand, it is of major value that numerous alternatives are available. But with each experimental hypothesis, special care should be taken to select the most suitable model. Despite all the experimental work done, the route by which pancreatic infection occurs and gives rise to septic complications and mortality has not yet fully been elucidated. Optimal prophylactic and treatment strategies are also still widely debated. In the future, animal models will undoubtedly provide increasing understanding of these subjects, but model-related drawbacks should always be kept in mind when designing a study or when interpreting results.

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