

Perioperative Treatment with Bactericidal/Permeability-Increasing Protein (rBPI₂₁) in Major Liver Surgery: A Concise Summary

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Abstract

Introduction: Major hepatic resections are still associated with considerable morbidity. Gut-derived bacteria and bacterial endotoxin are considered to play a central role in the pathophysiology of complications. Experimental studies suggest that bactericidal/permeability-increasing protein (BPI), which has both antibacterial and endotoxin-neutralising properties, can reduce postoperative complications. **Material and Methods:** A phase II, double-blind, placebo-controlled, multicentre, dose escalation trial was conducted in patients undergoing major liver resection, and clinical outcome, infectious complications, plasma amino acid patterns, coagulation and fibrinolytic cascade systems and neutrophil functions were compared between the two treatment groups and an extra group of patients undergoing major abdominal non-hepatic surgery. **Results:** Drug administration in this patient group was safe, and resulted in a significant reduction of infectious complications. Furthermore, beneficial effects were found in the postoperative amino acid ratio and fibrinolytic cascades, and rBPI₂₁ preserved leukocyte functions. **Conclusion:** Administration of rBPI₂₁ in patients undergoing major liver resection is well tolerated and results in improvement of both clinical and biochemical parameters.

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Key words: Amino acids, Clinical outcome, Coagulation and fibrinolysis, Endotoxin, Infections, Neutrophil function

Introduction

Primary and secondary hepatic malignancies constitute a major health problem. Hepatocellular carcinoma accounts for 90% of all primary hepatic malignancies in the world. While relatively uncommon in Western countries, it is the most prevalent malignant neoplasm in Southeast Asia, South Africa, and many other regions.¹ The liver is also the second most common site of metastatic diseases in the body after lymph nodes. Most liver metastases come from primaries in the gastrointestinal tract, pancreas, lung and breast, and nearly 50% of the patients with malignant tumours have metastases in the liver.² In Western countries, metastatic carcinoma are far more common than primary malignant tumours, as was demonstrated in a large United States cancer referral centre, where metastatic tumours to the liver were 41 times more common than primary hepatic carcinoma.²

Especially in colorectal cancer, the liver constitutes the primary site of metastatic disease.³ More than half of the patients undergoing curative resection for colorectal cancer will develop recurrent disease, and 80% of these patients will have metastatic disease in the liver.⁴ Patients with hepatic metastases have survival rates that vary from 6 to 18 months, and the single most important factor for length of survival is the extent of liver involvement.⁵

Considering convincing retrospective data, the grim natural history of both primary and secondary hepatic malignancies, and the improved safety of hepatic resection, it seems clear that patients should undergo hepatic resection if possible. In recent years, the early detection of lesions by improved imaging techniques as well as the newly developed surgical procedures such as the division of hepatic parenchyma by Cavitrol Ultrasonic Surgical Aspirator (CUSA) has lead to an increased number of

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patients suitable for surgical management of primary or metastatic neoplasms of the liver. Owing to these advances, together with the improved anaesthetic techniques and perioperative care, major hepatic resection in non-cirrhotic patients can be accomplished today with a mortality of less than 10%, as was indicated by recent studies.⁶⁻⁸ However, major hepatic resection remains associated with considerable morbidity.

Postoperative Complications and the Role of Endotoxin

Many complications can be directly related to surgical intervention, such as haemorrhage and bile leakage, but problems such as intra-abdominal sepsis, prolonged hepatic failure and respiratory distress may well be the result of impaired immune response after surgery. Generally, post-operative changes in the systemic immune response seem proportional to the degree of surgical trauma,⁹ which was confirmed by studies in which conventional surgery was compared with laparoscopic procedures. Recently, our group showed that laparoscopic surgery results in the preservation of immune functions, such as monocyte HLA-DR expression and plasma opsonic capacity.¹⁰⁻¹² Besides the prevention of postoperative infections, the preservation of an adequate immune response during and after surgery may also protect the patient against seeding and outgrowth of microscopic disease in cancer surgery. Because the surgically induced immunosuppression is multifactorial, with changes in both the humoral and the cellular response, there are many potential treatment modalities to abrogate this suppression. At the moment, several clinical strategies are being evaluated in our institute, such as nutritional interventions and the use of immune modulators like GM-CSF in colorectal cancer patients.^{13,14}

In liver surgery, it was also found that the larger the hepatic resection the greater the probability of postoperative complications.¹⁵ However, besides the normal immunosuppressive response seen in major surgery, liver resection is associated with an additional pathophysiological phenomenon, in which bacterial endotoxins play a major role. Under normal circumstances, only small amounts of endotoxin cross the intestinal barrier and are subsequently cleared by Kupffer cells, the resident macrophages of the liver and a major component of the mononuclear phagocytic system (MPS), which is the body's main clearing mechanism for bacteria and their endotoxin.¹⁶⁻¹⁹ However, after a major liver resection the number of Kupffer cells is strongly reduced, facilitating spill-over of gut-derived endotoxin into the systemic circulation.^{20,21} Indeed, endotoxin was demonstrated in plasma from patients undergoing a hemihepatectomy, whereas in plasma from patients undergoing other major abdominal surgery endotoxin was

not detectable.²² Endotoxin exerts its biological effects by activating immune cells such as monocytes and macrophages. After exposure to endotoxin, these cells readily produce and release several inflammatory mediators like TNF- α and IL-1, thereby inducing a systemic inflammatory response.²³⁻³⁰

In the liver, Kupffer cells are also capable of producing substantial amounts of inflammatory mediators in response to endotoxin. Some of these substances, such as the cytokines TNF- α and IL-6 and the growth factor HGF have a stimulatory effect on the liver's regenerative response, whereas other factors like IL-10 and TGF seem to inhibit the stress-related hepatic growth.³¹⁻⁴¹ The local inflammatory response in the remnant liver is a prerequisite for regeneration, but many of the inflammatory mediators that are produced during this process can also mediate liver injury.⁴²⁻⁴⁴

Some of the released cytokines also have a strong priming effect on neutrophils and induce the expression of leukocyte adhesion molecules on endothelial cells, which promotes an influx of activated neutrophils in the remnant liver.^{45,46} These neutrophils can generate tissue injury through the liberation of toxic oxygen radicals and proteolytic enzymes such as elastase and cathepsin G, which are highly cytotoxic to hepatocytes.⁴⁷ Further, the adherence of neutrophils to the endothelium leads to an impaired microcirculation, which results in the plugging of capillaries, the occlusion of hepatic sinusoids, and an aggravation of the hepatic injury and dysfunction.^{45,46,48} The role of neutrophils in the development of post-resection hepatic injury was clearly demonstrated by Pagetti et al⁴⁹ who showed that neutrophil depletion prior to injury greatly attenuated liver dysfunction. Thus, the process of liver regeneration after partial hepatectomy is a delicate balance between the stimulation of cell growth and the induction of tissue damage, and endotoxin can amplify the local inflammatory process in the liver because there is an increased sensitivity to endotoxin in the early phase after hepatectomy.⁵⁰

Further evidence for the adverse effects of endotoxemia after partial hepatectomy comes from studies in which various enteral anti-endotoxin agents were administered. The overall catabolic response as well as the postresection hepatic failure was significantly attenuated in a study in which the intestinal concentrations of bacteria and endotoxin were reduced through the administration of antibiotics, lactulose or cholestyramine. Further, in contrast to a high mortality rate in the control group no deaths occurred in the treated animals.^{51,52} Evaluation of *ex vivo* immune functions demonstrated that major liver resection resulted in a severe immunosuppression, as identified by the decreased proliferative response of splenic B and T lymphocytes together with reduced lipopolysaccharide (LPS) stimulated

production of TNF and IL-6 by splenocytes.⁵³ The preservation of these immune functions by binding intestinal endotoxin with cholestyramine further substantiated that translocation of gut-derived endotoxin is of major importance in the pathogenesis of complications following partial hepatectomy.⁵⁴

Bactericidal/Permeability-Increasing Protein (BPI)

In 1978, Elsbach and Weiss first isolated bactericidal/permeability-increasing protein (BPI), a natural human host-defence protein, with both bactericidal and endotoxin-neutralising properties.^{55,56} At first BPI was very unstable and therefore not suitable as an intravenous drug, but with the use of recombinant techniques smaller fragments of the protein were designed that had the same biological properties but were far more stable than the holo-protein.⁵⁷ Because of its bactericidal and endotoxin-neutralising properties, it was very conceivable that BPI could be effective in the prevention of endotoxin-mediated effects after liver resection and explorative studies were designed. In partial hepatectomised rats, the perioperative use of the recombinant BPI fragment rBPI₂₃ prevented endotoxemia and reduced the extent of liver failure. This was evidenced by lower plasma levels of intracellular liver enzymes and ammonia compared to controls and a greatly abolished infiltration of neutrophils in the remnant liver. The administration of rBPI₂₃ significantly increased the number of proliferating cells in the remnant liver, suggesting a preservative effect on the livers regenerative capacity.²¹ Further, the systemic effects of major liver resection such as the hypotensive response were reduced. Notably, this abolished systemic response coincided with a blunted cytokine response.⁵⁴

These studies clearly showed that endotoxemia after partial hepatectomy triggers a local as well as a systemic inflammatory response. Furthermore, the perioperative use of BPI in these animal studies was very beneficial in attenuating the detrimental biological effects of this inflammatory response.

The aim of this study was to investigate if treatment with BPI fragments could be effective in the prevention of endotoxin-mediated complications after liver resection in patients.

Material and Methods

The protocol was a phase II, double-blind, placebo-controlled, multicentre, dose escalation trial, conducted in patients undergoing a major liver resection. The protocol was approved by the Council for Medical Research of the Netherlands Organization for Scientific Research and by the institutional review boards or ethical review committees

of each participating institution, and informed consent was obtained from each patient before entry into the study. Patients were accrued in 5 academic hospitals and 2 specialised cancer institutes in the Netherlands, with patient eligibility for study entry primarily based on performance of a resection of ≥ 3 liver segments. All patients were randomly assigned in a 1:1 ratio to treatment with either rBPI₂₁ or placebo in an equal volume by 48-hour continuous intravenous infusion, starting about 1 hour prior to the start of resecting the liver. Mainly for safety reasons, the study was divided into two phases, a low dose phase with rBPI₂₁ infusion at a rate of 4 mg/kg/48 h and a high dose phase with an infusion rate of 8 mg/kg/48 h.

During the entire study period, the effect of rBPI₂₁ infusion was studied on clinical outcome and other factors, including the pharmacokinetics of rBPI₂₁, the plasma amino acid patterns, coagulation and fibrinolytic cascade systems and neutrophil functions.

Results

Pharmacokinetics of rBPI₂₁

Because animal studies have shown that under physiological conditions the liver is the most important organ for the clearance of BPI,⁵⁸ a reduction of liver mass could also result in a decreased clearance of rBPI₂₁, which could thereby reach toxic levels. Therefore, to address the question whether a major liver resection results in decreased clearance of rBPI₂₁ and subsequently higher plasma levels, the pharmacokinetics of rBPI₂₁ in rats following partial hepatectomy and in patients undergoing a major liver resection were investigated.

In rats, the pharmacokinetic properties of rBPI₂₁ were tested after liver resection of 60% and 80% with rBPI₂₁ administered in both groups at doses of 0.1, 1, and 10 mg/kg.

This study confirmed that the liver is the main organ for the clearance of rBPI₂₁, because a considerably delayed clearance was found in the 80% hepatectomised rats. However, in the 60% hepatectomised rats the decrease in clearance was very modest, suggesting a large reserve capacity of the remnant liver. This was also found in the low dose patients, where the clearance and steady state levels of rBPI₂₁ in patients undergoing partial hepatectomy were not significantly different from levels obtained in human volunteers. From these results it was concluded that the pharmacokinetics of rBPI₂₁ were not altered by partial hepatectomy sufficiently to adversely affect the clinical use of rBPI₂₁ in patients undergoing major liver resection, and the study was continued at the higher dose. Again, pharmacokinetic analysis showed no significant differences in clearance or steady state levels of rBPI₂₁ (Fig. 1).⁵⁹

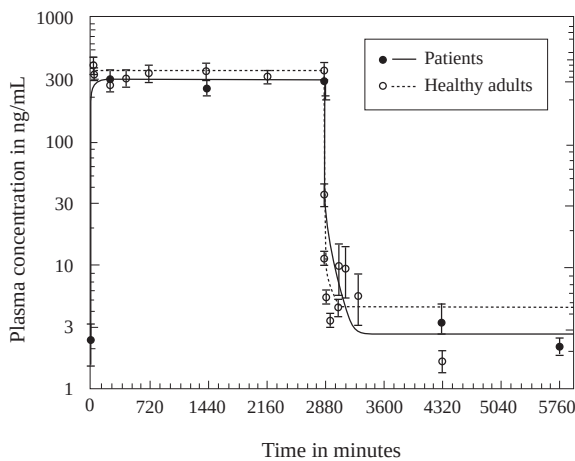


Fig. 1. Mean plasma concentration-time profile of 8 mg/kg rBPI₂₁ infused over 48 hours in healthy adults and partial hepatectomy patients. Average total BPI concentrations from hepatectomy patients (mean $\pm$ SE, $n = 10$) are shown. In addition, average total BPI concentrations from healthy adults (mean $\pm$ SE, $n = 4$) are shown superimposed on a curve derived from a two-compartmental pharmacokinetic model analysis of their average BPI concentrations.

Clinical Outcome and Infectious Complications

Most of the 12 patients that were treated with low dose rBPI₂₁ or placebo experienced a systemic inflammatory response, but the patients treated with rBPI₂₁ experienced slightly less (serious) adverse events (as defined by the International Congress of Harmonisation guidelines for "Good Clinical Practice"). Furthermore, they showed a reduction in hospital stay and stay in the intensive care unit, when compared with placebo-treated patients,⁶⁰ but because the results did not reach significance they did not allow any conclusions concerning efficacy.

However, it was concluded that rBPI₂₁ could be safely administered in patients undergoing major liver surgery, and again the higher dose phase was started.

From the 31 patients included during the high dose phase, 8 patients were randomised but not treated because the tumour was not resectable (5 patients) or could be removed by a small resection (3 patients). Thus, a total of 23 patients received treatment during the higher dose phase: 12 placebo and 11 rBPI₂₁ (Fig. 2). Both treatment groups were similar in terms of age, sex, primary tumour sites, comorbid factors and operative parameters such as the proportion of patients undergoing hemihepatectomy or extended hemihepatectomy, operative time, perioperative blood loss, red blood cell transfusions or fluid intake.

The predefined non-infectious complications, such as systemic inflammatory response syndrome (SIRS), hepatic failure, disseminated intravascular coagulation (DIC), acute respiratory distress syndrome (ARDS), acute renal failure, acute lung injury and hypotension, were equally distributed over the groups, and there were no significant differences

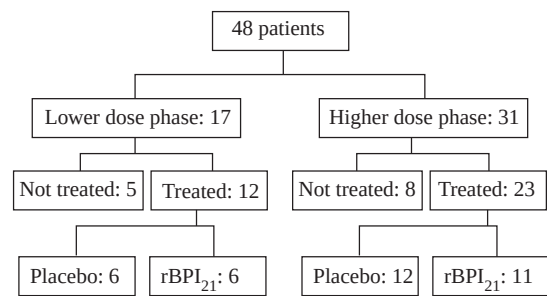


Fig. 2. Randomisation scheme.

in separate complications or the total number of non-infectious complications. However, even though the occurrence of hepatic failure was not significantly different between the high dose groups, the hepatic failure tended to persist longer in the placebo group, with 4 patients (33%) still having hepatic failure after day 14 compared to only 1 patient (9%) in the rBPI₂₁ group. The mean days alive and out of the hospital was 13 days for patients receiving rBPI₂₁ and 14 days for patients receiving placebo.

Whereas only 3 patients in the high dose rBPI₂₁ group (27%) experienced one or more predefined infectious complications, 8 patients in the placebo group (67%) experienced one or more infectious complications, a reduction of 60 %. The total number of infectious

TABLE Ia: INFECTIOUS COMPLICATIONS

Placebo	rBPI ₂₁
1. –	1. –
2. sepsis bacteraemia pneumonia parotitis cholangitis intra-abdominal infection	2. sepsis bacteraemia wound infection
3. sepsis urinary tract infection wound infection	3. –
4. wound infection	4. –
5. intra-abdominal infection	5. –
6. –	6. –
7. sepsis bacteraemia wound infection	7. –
8. sepsis pneumonia	8. sepsis urinary tract infection
9. pneumonia urinary tract infection	9. –
10. –	10. intra-abdominal infection
11. sepsis bacteraemia pneumonia	11. –
12. –	

TABLE Ib: TABLE OF INFECTIOUS COMPLICATIONS

No. of infectious complications	0	1	2	>2
Placebo patients (n)	4	2	2	4
rBPI ₂₁ patients (n)	8	1	1	1

$P = 0.035$ (Chochran-Mantel-Haenszel test for ordinal categorical data)

complications showed a significant difference between the two treatment groups ($P = 0.035$, Table I).⁶¹ This significance was particularly striking for pneumonia. While no patients in the rBPI₂₁ group developed pneumonia, 4 (33%) of the placebo patients had pneumonia.

Plasma Amino Acid Patterns

Severe hepatic failure as seen in hepatitis and cirrhosis and sometimes as a complication of extended liver resection may be accompanied by a hepatic encephalopathy and is characterised by a typical plasma amino acid pattern reflected by a decreased Fischer ratio.⁶²⁻⁶⁵ Because alterations in plasma amino acid pattern in patients undergoing a major liver resection have not been characterised and may be important in predicting postoperative morbidity and mortality, the present study evaluated plasma amino acid profiles in patients undergoing major liver resection receiving placebo or rBPI₂₁. In the placebo group, the Fischer ratio decreased significantly on the first and third postoperative day, however even though administration of both low and high dose rBPI₂₁ resulted also in a decreased Fischer ratio on day 1, no decrease was found on day 3. Furthermore, highly elevated alanine plasma levels were observed on day 1 in placebo-treated patients, whereas rBPI₂₁ prevented this elevation, and these alanine plasma levels on day 1 correlated well with the duration of postresection hepatic failure. From these results it was concluded that major liver resection results in a decreased Fischer ratio and a rise in alanine plasma levels, which correlated with the duration of the postresection hepatic failure. rBPI₂₁ improved the Fischer ratio and prevented the rise of alanine plasma levels, which could be of potential clinical significance.⁶⁶

Coagulation and Fibrinolytic Cascade Systems

Important complications of major liver resection associated with fatal outcome are hepatic failure and coagulation disorders, ultimately leading to disseminated intravascular coagulation.⁶⁷ It is well known that major liver resection is associated with elevated circulating endotoxin levels and in experiments with endotoxin challenge in humans, an activation of the coagulation and fibrinolytic cascade systems was seen³⁰ that could be inhibited by rBPI₂₃ infusion.⁶⁸ These data lead to the hypothesis that treatment with rBPI₂₁ could reduce

coagulopathy after major liver resection.

In this study the impact of major liver resection in patients on activation of coagulation and fibrinolysis in comparison to other major abdominal surgery without involvement of the liver was analysed and endotoxin-mediated effects were evaluated by perioperative treatment with low dose rBPI₂₁ ($n = 6$), high dose rBPI₂₁ ($n = 11$) versus placebo treatment ($n = 18$). Patients undergoing major abdominal surgery without involvement of the liver were included as an additional control group ($n = 13$).

The results showed no significant difference between the groups in coagulation and fibrinolytic system parameters like thrombin-antithrombin-III, plasmin- α 2-antiplasmin complexes, fibrinogen, plasminogen, α 2-macroglobulin and C1-inhibitor. However, it was found that patients who developed DIC had significantly higher plasma levels tissue type-plasminogen activator (t-PA), and that treatment with either low dose or high dose rBPI₂₁ largely prevented the increase in t-PA plasma levels. Because t-PA is released by endothelial cells, a reduced t-PA release by rBPI₂₁ most likely results from inhibition of endotoxin-mediated activation of endothelial cells. The association of significantly elevated levels of t-PA with the presence of DIC may indicate an important role of t-PA in the development of this serious postoperative complication. Therefore, the prevention of this elevation with rBPI₂₁ may have important clinical implications.⁶⁹

Neutrophil Function

Because elimination of pathogenic microorganisms during the direct postoperative period occurs mainly by phagocytes such as polymorphonuclear leukocytes (PMN) and monocytes, the development of postoperative infections may be due to an impaired function of these cells. Indeed, postoperative defects were reported for all aspects of leukocyte function, including chemotaxis,^{70,71} phagocytosis,^{72,73} oxidative burst,⁷⁴ enzyme release⁷⁵ and microbial killing.⁷⁶

Endotoxin is capable of inducing an enhanced expression of leukocyte and endothelial adhesion molecules and is a potent stimulator of the PMN oxidative burst *in vitro* and *in vivo*.⁷⁷⁻⁷⁹ Therefore, an endotoxin-induced change of PMN function could provide an explanation for the increased occurrence of infections and sepsis-like complications after major liver surgery. In order to investigate this hypothesis leukocyte oxidative burst and antigen expression were analysed in the patients undergoing a hemihepatectomy and receiving high dose rBPI₂₁-treatment or placebo. Further, to evaluate the specific influence of liver resection, an extra group of patients undergoing other major abdominal surgery served as controls.

The oxidative burst in the hemihepatectomised patients

receiving placebo and controls increased 2 hours after surgery, while in the rBPI₂₁-treated patients it decreased, resulting in significant differences between the groups. At day 1, neutrophil CD11b expression and monocyte CD14 expression in the rBPI₂₁-treated patients and controls and were significantly lower than that in the placebo group. At 2 hours, the CD16 expression in the placebo-treated patients was significantly higher than in the rBPI₂₁-treated patients and controls.

This study showed that patients undergoing major liver resection have an increased activation of leukocytes, when compared to other major abdominal surgery, which may contribute to the increased risk of infections in these patients. Administration of the endotoxin-neutralising agent rBPI₂₁ to hemihepatectomy patients was shown to partially preserve leukocyte functions and reduce leukocyte activation to the level of other non-hepatic abdominal surgery.⁸⁰

Conclusions

Despite improved surgical techniques and perioperative care, major liver surgery remains associated with substantial morbidity. From experimental work in the past, we know that bacterial endotoxins play a major role in the postoperative sequelae after liver surgery. In order to prevent postoperative complications, we used the endotoxin-neutralising agent rBPI₂₁ in patients undergoing major liver resection. This was one of the first clinical studies with rBPI₂₁ ever, and the very first study in surgical patients. The pharmacokinetic and immunogenicity results clearly proved the safety of the drug, and in the relatively small number of patients treated we found a statistically significant reduction of infectious complications. Furthermore, administration of rBPI₂₁ resulted in an improved Fisher ratio, and largely prevented the elevation of t-PA levels, two reactions that may also improve the clinical outcome. We further found that preservation of leukocyte functions may be the explanation for the reduced infection rate, but we do not know whether this was the result of a direct interaction of BPI and leukocytes (possibly receptor-mediated) or an indirect effect of BPI's endotoxin-neutralising power. We feel future clinical and experimental work should address these questions, in order to develop new treatment strategies.

In other fields of medicine, BPI has already proven its value. In children with severe meningococcal sepsis, rBPI₂₁ was beneficial in prevention of long-term disability.⁸¹ Further, in patients with haemorrhage due to trauma, BPI prevented pneumonia and acute respiratory distress syndrome.⁸² The results of our trial are very encouraging despite the low patient numbers, and we think rBPI₂₁ can be of significant value in liver surgery and transplantation in the future.

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