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ORIGINAL ARTICLE

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Selective bowel decontamination in elective liver transplantation: no improvement in endotoxaemia, initial graft function and post-operative morbidity

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Abstract Peri-operative endotoxaemia during liver transplantation has been linked to compromised graft function and infection. Selective decontamination of the digestive tract (SDD) could prevent endotoxaemia by eradicating Gram-negative bacteria from the intestine. In a randomized placebo controlled study we investigated the effects of endotoxaemia and the efficacy of SDD to prevent its occurrence. Thirty-one patients undergoing elective orthotopic liver transplantation received either SDD ($n=15$) or placebo ($n=16$), which was started at least 7 days before transplantation. Endotoxin levels were measured in blood peroperatively. Patients were scored daily for signs of liver dysfunction and infection. Endotoxaemia was neither associated with initial poor function nor any routine liver function test. Infections were more prominent in patients without endotoxaemia. SDD did not prevent endotoxaemia. Endotoxaemia does not affect post-operative graft function or the incidence of post-operative infections. SDD cannot prevent peri-operative endotoxaemia. Translocation of endotoxin may not be relevant in liver transplantation.

Keywords Endotoxin · Selective decontamination · Translocation · Liver transplantation · Initial poor function

Introduction

Although the treatment of choice for endstage liver failure for \many years, orthotopic liver transplantation still carries a considerable risk of complications. Initial graft function will be poor in 10%–20% of all transplants, and post-operative bacterial and fungal infection will develop in 40%–80% of the recipients [9, 13, 16].

Endotoxin is an integral part of the cell wall of aerobic Gram-negative bacilli. It is known for its capacity to induce the production of pro-inflammatory mediators such as TNF alpha and IL-1, which may lead to a systemic inflammatory response. In the gut it is present in large quantities and, since it can be demonstrated to be in portal blood in healthy volunteers, is probably absorbed to some extent. The liver plays an important role in clearing endotoxin from portal blood, mainly through its macrophages. Cirrhosis favours the translocation of endotoxin from enteric Gram-negative bacilli to the blood, which could explain why the haemodynamic profile in severe cirrhosis resembles that in Gram-negative sepsis [8]. During liver transplantation, intestinal ischaemia combined with temporary absence of hepatic clearance, might be expected to enhance such translocation of endotoxin. Indeed, endotoxaemia, the presence of endotoxin in peripheral blood during liver transplantation, has been described by various authors [7, 10, 12]. It is associated with initial poor function of the graft and seems to increase the rate of post-operative infection [7].

Selective decontamination of the digestive tract (SDD) is a procedure intended to prevent infection by the prophylactic eradication of Gram-negative aerobic

bacilli and yeasts from oropharynx, stomach and bowel, while preserving the normal anaerobic flora. Decontamination can be achieved with the enteral administration of various non-absorbable antibiotics. SDD can prevent endotoxaemia in certain animal models but its effect on endotoxaemia in human liver transplant patients has not been established definitively.

This study was designed to establish the presence or absence of endotoxaemia in human subjects undergoing liver transplantation, to assess the effect of endotoxaemia on initial graft function and post-operative infection, and to assess the power of SDD to prevent endotoxaemia.

Patients and methods

Study design

Data on endotoxaemia, graft function and post-operative infection were prospectively collected in 31 patients who had been randomized to treatment with either SDD or placebo. Randomization was performed by the hospital pharmacist. All other participants were kept unaware of the results of randomization. The study was approved by the local Ethics Committee.

Samples from portal vein, hepatic vein and arterial blood were taken at the start of the operation, 5 min before veno–venous bypass was started, 5 min before recirculation and 5, 30, 60 and 120 min after recirculation. Additional arterial blood samples were taken 12 h after recirculation. The arterial samples were taken from a canula in the radial artery.

All patients were scored daily for the presence of infection according to pre-defined criteria for the first 30 days following transplantation.

Graft function was assessed by determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, activated partial thromboplastin time, prothrombin time, antithrombin III levels (all at days 1 through 7) and lidocaine metabolism as indicated by the monoethylglycinexylidide (MEGX) test on the 1st 2 days after transplantation. Initial poor function and primary non-function were diagnosed according to criteria described by Ploeg and others [13].

Patients and controls

The trial included adult patients undergoing elective orthotopic transplantation of the liver in a university hospital in the Netherlands. Paediatric patients were excluded as were patients undergoing re-transplantation. Prophylactic treatment for spontaneous bacterial peritonitis with norfloxacin was a reason for exclusion, treatment with other antibiotic drugs was not. Patients were asked to participate in the study as soon as they were accepted for transplantation. If permission was obtained, patients were randomly assigned to groups receiving placebo or the SDD regimen by means of computer-generated numbers, and drug administration was commenced without delay. This regimen was continued until the day of transplantation. Post-operatively, a similar regimen of SDD or placebo was continued until the 30th post-operative day. Patients who had not received 7 full days of SDD or placebo before their transplantation were excluded from the study.

SDD regimen

Patients on SDD were put on a pre-operative regimen consisting of oral norfloxacin 400 mg once daily, and lozenges, containing 2 mg colistin, 1.8 mg tobramycin and 10 mg amphotericin B, four times daily. Post-operatively they received a suspension containing 200 mg colistin, 80 mg tobramycin and 500 mg amphotericin B, four times daily through a nasogastric tube, combined with an oral paste containing a 2% solution of the same drugs. If a nasogastric tube was no longer required, the suspension was replaced by tablets. Patients on placebo were on a similar regimen with placebo drugs.

Co-medication

Peri-operative antibiotic prophylaxis was started at the induction of anaesthesia and continued for 48 h. The standard regimen consisted of cefotaxime 1,000 mg every 8 h, combined with tobramycin 4 mg/kg once daily. In the presence of renal failure antibiotic prophylaxis consisted of imipenem 500 mg every 12 h. Anti-viral prophylaxis with aciclovir (200 mg every 6 h) was continued during the whole study period.

All patients received stress-ulcer prophylaxis with ranitidine. Immunosuppression consisted of a combination of prednisolone in tapering dose, cyclophosphamide (100 mg once daily for 7 days), azathioprine (125 mg once daily) and cyclosporin. Cyclosporin was started if creatinine clearance was ≥50 ml/min and adjusted to a whole blood through level of 250–300 ng/ml. Infections were treated with antibiotics at the discretion of the treating physician. There were no written restrictions on antibiotic policy connected with this study.

Microbiological studies

Stool cultures were obtained on the day of transplantation and (if available) on days 0, 2, 4, 6, 9, 11, 13, 16, 18, 20, 23, 25, 27 and 30. Decontamination was considered successful if less than 10^3 Gram-negative bacteria were cultured per cm^3 stool.

Endotoxin measurements

Samples were taken according to the method mentioned before. Blood was collected in Endotubes, kept on ice in order to avoid degradation and centrifuged at 200 *g* for 15 min at 4 °C. Platelet-rich plasma was frozen at −80 °C until measurements were performed.

Endotoxin levels were determined using the quantitative photometric limulus amoebocyte lysate assay (Kabi Diagnostics, Stockholm, Sweden), according to the manufacturers' instructions, in platelet-rich plasma. Plasma was stored at −80 °C. In order to avoid possible under-estimation of endotoxin levels, we also measured recovery of a known amount of endotoxin spiked to the platelet-rich plasma of each patient.

Definition of infection

Sepsis, septic syndrome and septic shock were diagnosed according to the definitions proposed by Bone and others [6]. Bacteraemia was defined as the presence of one positive blood culture; for coagulase-negative staphylococci (CNS) two positive blood cultures were required for the diagnosis of bacteraemia. In non-ventilated patients pneumonia was defined as a score of 7 or more on the Clinical Pulmonary Infection score, proposed by Pugin and coworkers [14]. In ventilated patients with a Clinical Pulmonary Infection score ≥7 a bronchoalveolar lavage was performed. A quantitative bacterial culture of $\geq 10^4$ cfu/ml in the bronchoalveolar lavage was considered to confirm the diagnosis of pneumonia. Abdominal infection was diagnosed in the presence of a body temperature > 38 °C in combination with a positive culture of ascites and leucocytes in ascites fluid $> 0.5 \times 10^6$/l. Abdominal infection was also considered to be present if fever disappeared after drainage of an abdominal abscess confirmed by CT, ultrasound or

surgery with a positive culture of drained material. Finally, a history of surgically and bacteriologically proven peritonitis, in the absence of an obvious other source of infection, also led to the diagnosis of abdominal infection. Cholangitis was diagnosed in the presence of a body temperature > 38 °C, chills, infected bile and an obstruction of the biliary tract. Urinary infection was diagnosed if, in the presence of a body temperature > 38 °C and $\geq 10^5$ bacteria/ml urine, no other obvious source of infection could be established. A wound infection was considered to be present if local signs of inflammation in a surgical wound were observed in combination with a positive culture of purulent discharge, which drained spontaneously or appeared after opening of the wound or during surgical exploration of the site of incision. Finally, a vascular catheter-related infection was diagnosed in the presence of a body temperature > 38 °C and one of the following conditions: (1) the same micro-organism was cultured from peripheral blood and from the catheter after its removal, with 15 colonies or more on the catheter (rolling plate method); (2) following removal of the line the patient's temperature dropped to < 38 °C within 24 h, without additional antibiotics and with a positive culture of the line (≥15 colonies).

Statistical analysis

The statistical analyses were performed with SPSS for Windows version 6.0 (SPSS, Chicago, Ill.). The Pearson chi-square test was used to compare the frequency of endotoxaemia between both groups. Student's *t*-test or the Mann-Whitney U test was used to compare the number of infections, the occurrence of initial poor function and parameters of post-operative morbidity.

A P value < 0.05 was considered to imply statistical significance.

Results

Patients on SDD ($n = 15$) and patients on placebo ($n = 16$) were well matched with respect to baseline clinical, demographic and health-status measurements (Table 1).

All patients with SDD were successfully decontaminated throughout the test period. Median duration of decontamination was 117 days (range: 7–324 days) before transplantation.

Endotoxaemia was detected in three patients receiving SDD (20%) and in six patients receiving placebo (38%) (Fig. 1). This difference was not statistically significant (Pearson chi-square test). In six patients endotoxaemia became apparent during the anhepatic phase (two SDD patients and four on placebos) and resolved, in all but one, within 1 h after reperfusion. In three patients endotoxaemia occurred approximately 1 h after reperfusion. This resolved within 1 h, since at 2 h after reperfusion no endotoxaemia was present. Differences between portal, hepatic vein and systemic arterial blood endotoxin concentrations were not observed at any moment during transplantation (Wilcoxon matched-pairs signed-rank test).

The data on post-operative infections are presented in Table 2.

Surprisingly, the overall number of post-operative infectious episodes was higher in the group without endotoxaemia than in patients who did have detectable quantities of endotoxin in their blood during surgery ($P < 0.03$). The same holds true for abdominal infections.

Graft function and ICU stay are shown in Table 3. No significant differences were observed in the incidence

Table 1. Demographic data

Characteristic	SDD	Placebo
Gender (male/female)	9/6	9/7
Age (years)	43 (±10)	44 (±14)
Child-Pugh score (A/B/C)	3/7/5	3/2/10
UNOS score (1/2/3/4)	10/1/2/1	11/0/3/2
Total ischaemia time (min)	735 (±205)	775 (±220)
Disease		
Cirrhosis e.c.i.	3	4
Primary sclerosing cholangitis	2	4
Primary biliary cirrhosis	2	1
Hepatitis C		3
Alcoholic cirrhosis	1	1
Familial amyloid polyneuropathy		2
Chronic active hepatitis	1	1
α-1-Antitrypsin deficiency	1	1
Other	4	

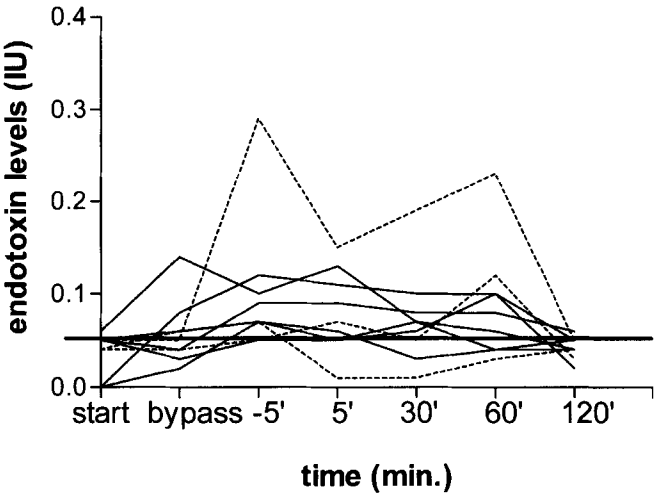

Fig. 1. Patterns of endotoxin levels in arterial blood in individual patients who experienced endotoxaemia at some point during transplantation. *Dotted lines* represent patients receiving SDD, *straight lines* depict patients receiving placebo. *The thick horizontal line* represents the cut-of point of 0.05 IU endotoxin/ml. Point zero was time of reperfusion

Table 2. Post-operative infections

Type of infection	Endotoxaemia ($n = 9$)	No endotoxaemia ($n = 22$)
Pneumonia	0 (0%)	3 (13%)
Bacteraemia	4 (44%)	11 (50%)
Cholangitis	1 (11%)	4 (18%)
Abdominal infection	1 (11%)	12 (55%)*

*$P < 0.03$

Table 3. Graft function and ICU stay. No significant differences were noted between the assessed parameters

Parameter	Endotoxaemia ($n=9$)	No endotoxaemia ($n=22$)
No. of patients with initial poor function (%)	0 (0%)	4 (18%)
Mean prothrombin time (SD) (s)		
Day 1	23 (±6)	20 (±4)
Day 2	21 (±3)	20 (±6)
Day 3	18 (±1)	18 (±3)
Day 4	18 (±1)	17 (±2)
Day 5	17 (±2)	17 (±3)
Day 6	17 (±2)	17 (±3)
Day 7	19 (±2)	17 (±4)
Mean AST (SD) (IU)		
Day 1	500 (±380)	700 (±540)
Day 2	400 (±300)	700 (±800)
Day 3	180 (±100)	440 (±570)
Day 4	95 (±35)	160 (±105)
Day 5	70 (±30)	100 (±45)
Day 6	65 (±25)	100 (±110)
Day 7	90 (±55)	90 (±65)
Mean MEGX increase (SD) (μg/l)		
Day 1	43 (±26)	56 (±24)
Day 2	68 (±38)	71 (±48)
Median ICU stay	7 days	13 days

of initial poor function, any of the assessed liver function tests and median ICU stay, between endotoxaemic and non-endotoxaemic patients.

Discussion

Endotoxaemia during the anhepatic phase of liver transplantation was demonstrated by Starzl's group as early as 1989 [11, 12, 17]. In animal models as well as in human transplant patients they could establish a correlation between systemic endotoxin levels at the end of the anhepatic phase and the occurrence of post-operative complications and death. The need for platelet transfusion and post-operative ventilatory support was lower in patients with low systemic endotoxin levels. They also proposed that endotoxaemia could be a cause of graft loss, since patients with primary non-function had high levels of endotoxin in their blood. The same group could show that detectable endotoxin levels were associated with decreased post-operative renal function [18]. Fugger and co-workers could not confirm these results: peripheral endotoxaemia during liver transplantation was unpredictable and not related to graft function [7]. Circulating endotoxin levels during liver transplantation were also evaluated prospectively by Blanot et al. [4, 5]. Fluctuations of the plasma endotoxin levels during the procedure were low. A relationship between the level of endotoxaemia and the occurrence of the post-reperfusion syndrome could not be established. In 20 patients undergoing liver transplantation Steininger et al. [15] found endotoxaemia before and after transplantation in four, pre-operative endotoxaemia disappearing during transplantation in seven and no endotoxaemia in nine patients. The only patient with severe endotoxaemia showed a significant transhepatic concentration difference in endotoxin concentration, with high endotoxin levels measured in the hepatic vein (151 ng/l). It was concluded that in this patient the liver was an endotoxin-producing organ. The patient went on to develop graft dysfunction and severe abdominal infection. In a study by Bion and co-workers to assess the effect of SDD in patients undergoing a liver transplant, peripheral endotoxaemia was observed in approximately 60% of the patients [3]. No correlation was found between the presence of peripheral endotoxaemia and the need for ventilatory support, re-transplantation or the development of multiple organ dysfunction.

The results of our study also fail to confirm the original reports from the Starzl group. Endotoxaemia was present in only 29% of the patients, and no correlation was found with initial graft function. The low incidence of endotoxaemia in either of our groups might be explained by the assay used to determine endotoxin concentrations. Another explanation might be the patient population: patients with ascites are known to have an increased incidence of raised endotoxin levels. Secondly, surgical technique might play a role, though in our group it made no difference whether splanchnic decompression was performed by a bypass or not.

Surprisingly, in our study, infections were statistically more likely to occur in the non-endotoxaemic group, a finding for which no obvious explanation can be given.

SDD has been shown to reduce portal endotoxaemia in an animal model [1]. It can also attenuate liver injury following transplantation. In a non-blind study in

humans Bion and co-workers found no difference in endotoxaemia between SDD patients and controls [3]. SDD was started between 12 and 24 h before surgery, which might have been insufficient to achieve a meaningful decrease in endotoxin load during the transplantation. However, in our own study, which was placebo controlled and where patients had a minimum of 7 days of decontamination before surgery, endotoxaemia was not prevented by SDD either. Possible explanations might be that despite our cut-off point of successful decontamination (fewer than 10^3 Gram-negative bacteria per cm^3 stool) being reached, the remaining Gram-negative bacteria caused endotoxaemia. Also, the digestive tract might not be the sole source of circulating endotoxins, especially if one keeps in mind that in our study no difference was found between portal and hepatic vein endotoxin concentrations. Other possible explanations for this finding might be that endotoxin clearance by the liver is not a first pass effect but is achieved more slowly and continuously. On the other hand, it was technically impossible to perform punctures in the portal and hepatic vein at *exactly* the same time, although both samples were taken within seconds of each other. This might have caused a problem in identifying very small differences in concentrations between portal and hepatic vein endotoxin concentrations.

It thus appears that the data on the relevance of endotoxaemia during liver transplantation are conflicting. Peripheral endotoxaemia does occur in a number of patients but most studies have failed to link endotoxaemia consistently with post-operative complications. Endotoxaemia cannot be prevented by SDD, even if SDD is started early enough to achieve elimination of Gram-negative aerobic bacilli at the time of surgery. In our view there is insufficient evidence to accept the concept of enhanced translocation of endotoxin during liver transplantation as a relevant mechanism of disease in these patients. However, endotoxin might be more important in recipients receiving a liver from a donor with endotoxin either circulating in the blood or pooled in the graft. Bismuth and co-workers have shown in a rat model that endotoxin administered to a liver graft donor can be transferred to the recipient [2]. A transfer of endotoxin might thus be more relevant to the recipient than presumed translocation from the gut.

References

1. Arai M, Mochida S, Ohno A, Arai S, Fujiwara K (1998) Selective bowel decontamination of recipients for prevention against liver injury following orthotopic liver transplantation: evaluation with rat models. Hepatology 27:123–127
2. Azoulay D, Astarcioglu I, Lemoine A, Dennison A, Mathieu D, Saulnier C, Chatenoud L, Reynes M, Bismuth H (1995) The effects of donor and recipient endotoxemia on TNF alpha production and mortality in the rat model of syngenic orthotopic liver transplantation. Transplantation 59:825–829
3. Bion JF, Badger I, Crosby HA, Hutchings P, Kong KL, Baker J, Hutton P, McMaster P, Elliott TS (1994) Selective decontamination of the digestive tract reduces Gram-negative pulmonary colonization but not systemic endotoxemia in patients undergoing elective liver transplantation. Crit Care Med 22: 40–49
4. Blanot S, Gillon MC, Ecoffey C (1993) Circulating endotoxins during orthotopic liver transplantation and postreperfusion syndrome. Lancet 342: 859–860
5. Blanot S, Gillon MC, Lopez I, Ecoffey C (1995) Circulating endotoxins and postreperfusion syndrome during orthotopic liver transplantation. Transplantation 60:103–110
6. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RMH, Sibbald WJ (1992) Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Chest 101:1644–1655
7. Fugger R, Hamilton G, Steininger R, Mirza D, Schulz F, Mühlbacher F (1991) Intraoperative estimation of endotoxin, TNF alpha, and IL-6 in orthotopic liver transplantation and their relation to rejection and postoperative infection. Transplantation 52:302–306
8. Guarner C, Soriano G, Tomas A, Bulbena O, Novella MT, Balanzo J, Vilardell F, Mourelle M, Moncada S (1993) Increased serum nitrite and nitrate levels in patients with cirrhosis: relationship to endotoxemia. Hepatology 18:1139–1143
9. Makowka L, Gordon RD, Todo S, Ohkohchi N, Marsh JW, Tzakis AG, Yokoi H (1987) Analysis of donor criteria for the prediction of outcome in clinical liver transplantation. Transplant Proc 19:2378–2382
10. McNicol L, Liu G, Hurley J, Hardy KJ, Jones RM (1993) Endotoxin and tumor necrosis factor levels and hemodynamics during human liver transplantation. Transplant Proc 25:1828–1829
11. Miyata T, Todo S, Imventarza O, Ueda Y, Furukawa H, Starzl TE (1989) Endogenous endotoxemia during orthotopic liver transplantation in dogs. Transplant Proc 21:3861–3862
12. Miyata T, Yokoyama I, Todo S, Tzakis A, Selby R, Starzl TE (1989) Endotoxaemia, pulmonary complications, and thrombocytopenia in liver transplantation. Lancet 2:189–191
13. Ploeg RJ, D'Alessandro AM, Knechtle SJ, Stegall MD, Pirsch JD, Hoffmann RM, Sasaki T, Sollinger HW, Belzer FO, Kalayoglu M (1993) Risk factors for primary dysfunction after liver transplantation – a multivariate analysis. Transplantation 55:807–813
14. Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter PM (1991) Diagnosis of ventilator associated pneumonia by bacteriological analysis of bronchoscopic and non-bronchoscopic blind bronchoalveolar lavage fluid. Am Rev Respir Dis 143:1221–1229

15. Steininger R, Fugger R, Hackl W, Hamilton G, Langle F, Herbst F, Gnant M, Sautner T, Rogy M, Schulz F et al. (1990) Immediate graft function after OLT clears endotoxin. Transplant Proc 22:1544–1546
16. Wade JJ, Rolando N, Hayllar K, Philpott-Howard J, Casewell MW, Williams R (1995) Bacterial and fungal infections after liver transplantation: an analysis of 284 patients. Hepatology 21:1328–1336
17. Yokoyama I, Todo S, Miyata T, Selby R, Tzakis AG, Starzl TE (1989) Endotoxemia and human liver transplantation. Transplant Proc 21:3833–3841
18. Yokoyama I, Gavaler JS, Todo S, Miyata T, van Thiel DH, Starzl TE (1995) Endotoxemia is associated with renal dysfunction in liver transplantation recipients during the first postoperative week. Hepatogastroenterology 42:205–208