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β-Glucan Reflects Liver Injury After Preservation and Transplantation in Dogs

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ABSTRACT Graft failure and extrahepatic organ complications, which frequently develop after transplantation, may be related to inflammatory mediators stimulated by endotoxin (ET). The role of endotoxemia after liver transplantation is controversial and may depend upon differences in the ET assay method used in the various contradicting studies. While the standard *Limulus* amebocyte lysate (LAL) is reactive for ET and β-glucan, a novel turbidimetric assay method enables separate determinations of ET and β-glucan. Beagle dogs undergoing orthotopic liver transplantation were divided into two groups. In Group I ($n = 6$) the grafts were transplanted immediately and in Group II ($n = 6$) grafts were preserved for 48 h in University of Wisconsin (UW) solution. Animals received cyclosporine immunosuppression and were followed for 14 days. Daily measurements of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were performed. Samples for ET and β-glucan measurement were collected serially and processed using the turbidimetric assay method. While no graft failure was seen in Group I, three of six Group II animals died from graft failure within 1 day after transplantation. Preservation and reperfusion injury was much more severe in the Group II grafts than in Group I grafts. While endotoxemia could not be detected, postoperative β-glucan levels (undetectable pretransplant) were seen in both groups. β-Glucan levels were much higher in Group II grafts than in Group I grafts, and correlated with the severity of liver damage. In conclusion, this study shows that β-glucann, instead of ET, appears during the early posttransplant period. We believe that posttransplant elevation of β-glucan is related to liver damage, especially endothelial damage by preservation and reperfusion.

KEYWORDS β-glucan, canine, ischemia, liver, organ preservation, reperfusion injury, transplantation

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Although orthotopic liver transplantation has become a standard treatment for patients with end-stage liver disease, graft failure and extrahepatic organ complications frequently develop after

transplantation [1]. These complications may be related to inflammatory mediators stimulated by endotoxin (ET) escaping hepatic filtration. We [2–4] and others [5–7] have previously reported postoperative endotoxemia, in which ET levels correlated with the severity of liver injury and clinical outcome. However, endotoxemia after liver transplantation was not always seen in some studies [8–10]. Differences in the ET assay method appear to cause the discrepancy. Previously, we measured ET by a chromogenic standard *Limulus* amebocyte lysate (LAL) assay. The standard LAL is not specific for ET because it is also reactive to β-glucan [11]. In this study, endotoxemia after canine liver transplantation was reexamined by a novel turbidimetric assay method that enables separate determinations of ET and β-glucan.

METHODS

This study was approved by the University of Pittsburgh's Institutional Animal Care and Use Committee. Healthy female beagle dogs, weighing 9 to 11 kg, were used. Orthotopic liver transplantation with the use of a veno-venous bypass was performed by the method described previously [12]. The animals were divided into two experimental groups: Group I ($n = 6$), undergoing immediate liver transplantation, and Group II ($n = 6$), receiving livers that were preserved for 48 h with University of Wisconsin (UW) solution (ViaSpan, DuPont, Wilmington. DE). A modification of the simple cold storage method [13] was used for preservation of Group II livers. After in situ flushing with UW solution and occlusion of the vena cava and hepatic artery, grafts were flushed with an additional 30% of UW solution (1% accounts for 1 ml/1 g graft weight) through a portal venous cannula. Animals were given oral cyclosporine, 20 mg/kg, from the next morning for posttransplant immunosuppression and followed for 14 days. Daily measurements of aspartateaminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were performed until sacrifice.

ET and β-Glucan Measurement

Before starting the recipient operation, at the end of the anhepatic phase (5 min before graft reperfusion), and at 0.5, 1, 3, 6, 12, and 24 h after transplantation, 5 ml of the peripheral venous blood was drawn in a sterile fashion into a pyrogen-free plastic syinge containing 50 IU heparin. The samples were transferred immediately into pyrogen-free test tubes, placed on ice, and centrifuged at 4°C at 3000 rpm for 40 s. The separated platelet-rich plasma was stored at −80°C. Measurements of ET and β-glucan were automatically performed in duplicate by a kinetic turbidimetric method using ES buffer and HS buffer, respectively, and a toxinometer (ET-251, Wako Pure Chemical Industries Ltd., Osaka, Japan). The ES buffer is an endotoxin-specific LAL reagent with inactivated glucan factor (G-factor). The HS buffer is specific for β-glucan and eliminated ET activity from the assay. Samples for both assays underwent detergent dilution and heat inactivation prior to measurement. The minimum detection limit was 1.4 pg/ml for ET and 0.19 ng/ml for β-glucan. Results are expressed as the mean ± SD. One-way analysis of variance was used to compare group means using the software packages Statistica (StatSoft, Tulsa, OK) and SPSS (SPSS, Inc., Chicago).

RESULTS

Survival, maximum AST, ALT, and LDH values, peak ET and β-glucan levels, and cause of mortality in Group I and Group II animals after liver transplantation are shown in Table 1. In Group I, 2 of 6 died before 14 days; one died on postoperative day (POD) 1 from pulmonary hemorrhage and the other was lost to intussusception on POD 6. No graft failure was seen in this group. In contrast. 3 of 6 Group II animals died from graft failure within 1 day after transplantation. The remaining 3 survived for 14 days. Liver enzyme levels indicated that preservation and reperfusion injury was much more severe in the Group II grafts than in grafts that underwent immediate transplantation. While endotoxemia could not be detected in either Group I animals or Group II animals at any stage of liver transplantation, postoperative β-glucan levels were seen in both groups. [β-Glucan levels, undetectable before the operation, increased significantly at the end of the anhepatic phase in both groups (Figure 1).] After graft reperfusion, β-glucan levels of Group I

TABLE 1 Results of orthotopic liver transplantation in dogs

		Maximum value			Peak levels		
Experiments	*Survival (days)*	*AST (IU/L)*	*ALT (IU/L)*	*LDH (IU/L)*	*Endotoxin*	*β-Glucan (ng/ml)*	*Cause of death*
Group I							
1	6	849	845	570	ND	7.12	Intussusception
2	14	301	805	496	ND	3.5	Scheduled sacrifice
3	14	1495	1560	1020	ND	2.2	Scheduled sacrifice
4	1	1900	2285	2435	ND	4.53	Hemorrhage lung
5	14	1090	1130	575	ND	6.17	Scheduled sacrifice
6	14	1800	568	717	ND	4.15	Scheduled sacrifice
Group II							
1	14	4960	5160	5260	ND	20.09	Scheduled sacrifice
2	14	3212	3120	1940	ND	15.29	Scheduled sacrifice
3	14	5070	6150	2850	ND	10.22	Scheduled sacrifice
4	1	9000	10,080	3780	ND	20.69	Graft failure
5	0.5	19,240	18,000	6020	ND	26.47	Graft failure
6	1	10,116	6984	3420	ND	24.83	Graft failure

Note. ND, not detected; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase.

animals peaked at 0.5 to 1 h (mean peak level 4.6 ± 1.8 ng/ml), and became undetectable after 6 to 24 h. In Group II, the postreperfusion increase of β-glucan was enhanced (mean peak level 19.6 ± 6.0 ng/ml) and lasted for a longer period than in Group I, with peak levels 4 to 5 times higher at 3 and 6 h after repedusion. β-Glucan of the 3 failed grafts remained elevated, and β-glucan levels of the 14-day survivors showed some reduction 24 h after reperfusion.

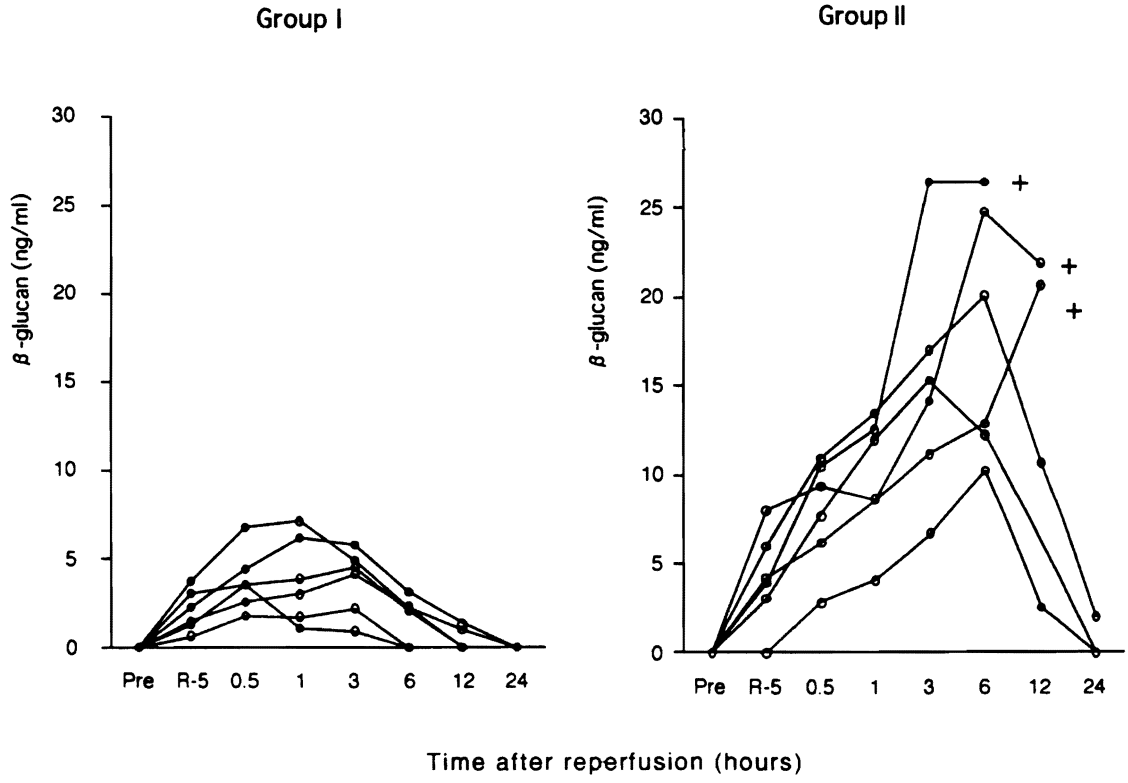

FIGURE 1 β-Glucan levels after transplantation for Group I and Group II. R-5 represents 5 min before reperfusion; +, failed grafts.

DISCUSSION

ET causes gelation and clotting of LAL extracted from horseshoe crab hemocytes. Since Bevin and Bang [14] first found this phenomenon, LAL has been used, as a *Limulus* test, to detect ET in blood and pharmaceutical products. However, due to problems of sensitivity and quantification with the *Limulus* test, new ET assay methods, such as chromogenic analysis [15], turbidimetry [16], and enzyme-linked immunosorbent assay by monoclonal antibody [17], have recently been developed. These methods have enabled measurement of ET at very low concentrations (picogram), and revealed endotoxemia in patients with sepsis and liver diseases [15, 16]. However, when standard LAL was used for measurements, it was found that β-glucan interfered with the results when present in the assay system. Iwanaga et al. [18] has reported that the coagulation cascade of standard LAL is initiated through either ET-sensitive factor C or β-glucan-sensitive factor G. Therefore, either the modified LAL that eliminates factor G or the modified assay method that inactivates factor G is necessary to measure ET specifically.

In our previous study using a chromogenic assay with standard LAL, we reported endotoxemia during the perioperative period of liver transplantation in experimental animals [2] and in patients [3, 4]. ET levels correlated with the severity of liver injury, primary graft nonfunction, overall graft loss, OKT3 use, and extrahepatic organ dysfunction. From our previous results, we speculated that inflammatory mediators stimulated by ET may cause hepatic and extrahepatic complications after liver transplantation. However, in this study, a turbidimetric analysis showed no endotoxemia in any of the experimental animals even though we used the same operative procedure and postoperative management as in the previous study. The differences in these observations may be attributed to the ET assay method. The standard LAL ET assay is incapable of differentiating between ET and β-glucan, while the turbidimetric method is specific for endotoxin. The standard LAL was used in the previous study, because it was the only method available at that time to measure ET; however, a modified LAL assay has provided results compatible with the turbidimetric method in experiments testing the recovery rate of ET, as shown in our laboratory (unpublished data) and by others [16].

The present study showed the presence of β-glucan, instead of ET, during the early postoperative period. β-Glucan was undetectable before the operation, appeared at the anhepatic phase, and elevated transiently after reperfusion of the graft in both groups. Postoperative elevation of β-glucan was higher after 48-h liver preservation than with immediate transplantation, and correlated with the severity of liver damage.

β-Glucan is one of the most important constituents of the cell wall of fungi [19]. It protects fungi from deformity, desiccation, and environmental alterations. In addition, β-glucan-like activity has been demonstrated with hemodialyzing membranes made of cellulose [20] and in human plasma [21]. As the source of β-glucan appearing at perioperative of liver transplantation, four possibilities could be considered: (1) coexistence of fungal infection, (2) impaired hepatic filtration or metabolism of β-glucan of enteric origin, (3) veno-venous bypass system, and (4) liberation from the hepatic graft. Fungal infection is unlikely because normal healthy animals were subjected to the experiments and no β-glucan was detected before operation. β-Glucan of enteric orign is also unlikely since endotoxin, which is much more abundant than β-glucan in the enteric lumen, was not detected in this study. The veno-venous bypass system could, in part, be a source of β-glucan because similar levels of β-glucan were measured in both groups during the anhepatic phase. β-Glucan-like substance was reported to increase also during cardiopulmonary bypass [7]. However, bypass system origin cannot explain the entire profile of β-glucan in this experiment, in particular marked elevation of β-glucan in Group II after reperfusion. Although β-glucan levels in portal venous blood and hepatic venous blood were not measured, we believe that posttransplant elevation of β-glucan is related to liver damage, especially endothelial damage by preservation and reperfusion. Kikuchi et al. [22] found β-glucan-like activity in rat organ tissues, mostly in vessels. They considered that when vascular damage occurred, β-glucan-like substance was released from endothelial cells into the circulation.

Endothelial cells of the microvasculature have been shown to be a main target of preservation and reperfusion injury of the liver [23]. Whatever the reasons, the results from this study warrant reevaluation of endotoxemia in clinical liver transplantation to determine the cause of hepatic and extrahepatic complications and to improve their prevention and management strategy.

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