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ORIGINAL ARTICLE

Impact of hepatic clearance of endotoxin using endotoxin activity assay

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Abstract
Background Endotoxin (Et) in the portal vein blood is processed by the hepatic reticuloendothelial system, and therefore, it is possible that the hepatic clearance of Et may become a biological index for liver function. In this study, Et levels of preoperative peripheral and portal vein blood at the time of liver transplantation (LT) were measured in order to study the meaning.
Methods The study population comprised 19 patients in whom pediatric living donor LT was performed. In the preoperative peripheral and the portal vein blood at the time of LT, we measured Et activity (EA) by the Et activity assay (EAA) and the Limulus amebocyte lysate (LAL) method.
Results The preoperative peripheral vein blood showed a low EA in all cases. In the EA of the peripheral and the portal vein blood, the latter showed a significantly high level ($p = 0.049$). With the LAL method, 5.3% (2/38) of patients were positive for Et.
Conclusions The EAA is considered to be superior to the LAL method for the detection of Et, even in low endotoxinemia, and is also capable of elucidating the Et kinetics by accurately reflecting hepatic clearance.

Keywords Endotoxin activity assay · Limulus amoebocyte lysate · Endotoxin · Hepatic clearance · Liver transplantation

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Introduction

Endotoxin (Et) in the portal vein blood is processed by the hepatic reticuloendothelial system. Decreased liver function and the inability to process Et may cause endotoxinemia [1, 2, 3]. Therefore, it is possible that the hepatic clearance of Et may become a biological index for liver function.

In the past, Et was frequently measured using the limulus amebocyte lysate (LAL) method, but there were problems regarding the time spent on measurement results, the reliability of measured values, etc. [4, 5, 6]. In recent years, the Et activity assay (EAA) capable of rapidly measuring the neutrophil activity by a chemiluminescence method has been developed [6, 7], and the assay's efficacy has also been recognized [8, 9].

In this study, preoperative Et levels in peripheral and portal vein blood at the time of liver transplantation (LT) were measured in order to study the meaning of measurement by EAA, and our findings are reported herein.

Materials and methods

Subjects

This study was performed on 19 patients in whom pediatric living donor LT was performed at our department between

April 2010 and January 2011. There were nine males and ten females with a median age of 1.0 year (range 0.2–19.1 years) and a median body weight of 8.6 kg (range 3.2–49.7 kg). The original diseases were biliary atresia (BA) in 13 cases, ornithine transcarbamylase deficiency (OTCD) in 3, biliary liver cirrhosis (LC) in 1, fulminant hepatic failure (FHF) in 1, and primary sclerosis cholangitis (PSC) in 1. Approval to conduct this study was obtained from the Ethics Committees of Jichi Medical University.

Methods

In the preoperative peripheral and the portal vein blood at the time of LT, Et activity (EA) by EAA and by the LAL method was measured. Beta-D glucan and NH_3 levels were also simultaneously measured.

EAA

The EAA [6, 7] (EAA™, Spectral Diagnosis Inc.) is a rapid in vitro diagnostic test that utilizes a specific monoclonal antibody to measure the EA in EDTA whole blood specimens. This assay uses the biological response of the neutrophils in a patient's blood to the immunological complex of Et and exogenous antibody as a measure of Et activity. The assay reacts specifically with lipopolysaccharide (LPS) of Gram-negative bacteria and does not cross-react with cell wall constituents of Gram-positive bacteria and other microorganisms. A 1.2-ml sample of whole blood was drawn into an Et-free EDTA blood collection tube. Blood samples were maintained at room temperature and all samples were assayed $\leq$3 h of collection.

The EAA is based on the reaction of Et with a specific anti-Et antibody raised against the lipid A of *Escherichia coli* J5. Complement proteins opsonize the Et–antibody complex. The opsonized immune complex primes neutrophils in the blood to enhance their respiratory burst in response to zymosan. The respiratory burst of the neutrophils yields oxidants that react with luminol in the reaction mixture to emit chemiluminescence. The chemiluminescence can then be detected in a photon counting luminometer (SmartLine TL, Berthold).

A basal activity measurement (Tube 1) in the absence of the specific anti-Et antibody measures the nonspecific oxidative burst of the patient's neutrophils. An additional control measurement including the specific anti-Et antibody and an excess of exogenous Et (Tube 3) measures the maximum oxidative burst of the patient's neutrophils. The test measurement (Tube 2) includes the specific antibody to measure the neat level of EA. All three tubes are incubated at 37°C for 14 min and are assayed in triplicate. The EA level is calculated by normalizing the chemiluminescence in the test sample (Tube 2) against the maximum chemiluminescence (Tube 3), while correcting both measurements for the basal activity chemiluminescence (Tube 1).

The EA level of 0.4 is approximately equivalent to an Et concentration of 25–50 pg/mL, and a level of 0.6 is approximately equivalent to an LPS concentration of 100–200 pg/mL of *E. coli* 055:B5 LPS.

For EA, <0.4 was determined to be a low EA, 0.4–0.6 intermediate EA, and >0.6 high EA.

LAL method

The most commonly used diagnostic test—the chromogenic LAL assay [10]—is based on the ability of Ets to induce coagulation of the hemolymph of the horseshoe crab, *Limulus polyphemus*.

The measurement is conducted utilizing the reaction wherein Et and a Limulus reagent, derived from horseshoe crab hematocytes, specifically coagulate. A clotting enzyme is activated at the final stage of the Limulus cascade and coagulin appears. There is formation of insoluble coagulin polymers by further polymerization, followed by formation of a gel. Changes in turbidity from this gelation are quantitatively measured.

Pretreatment of diluting and heating at 70°C for 10 min and using high-sensitive turbidimetric time analysis, the gelation reaction time of a Limulus reagent was treated as changes in the amount of transmitted light of the reaction solution. With the reaction time until the amount of transmitted light of the reaction solution decreasing by a certain ratio as the gelation time, the Et level was measured based on the relationships with Et concentrations.

Statistical analysis

The EA by EAA was compared between the peripheral and the portal vein blood using the Mann–Whitney *U* test. In the comparison, differences at $p < 0.05$ were considered to be significant.

Results

With the LAL method, 5.3% (2/38) cases were positive, and one case (LC) of the preoperative peripheral venous blood and one case (BA) of portal vein blood were positive (Table 1), but they both showed a low EA by the EAA.

The preoperative peripheral vein blood showed a low EA in all cases, and the portal vein blood at the time of LT showed a low EA in 16 cases and an intermediate EA in 3 cases (OTCD, LC, BA) (Table 2). In the EA of the peripheral and the portal vein blood, the latter showed a significantly high level ($p = 0.049$) (Fig. 1).

Table 1 Data of the Et concentrations by the LAL method

Case	Original disease	Peripheral vein blood	Portal vein blood
1	OTCD	<0.8	<0.8
2	BA	<0.8	2.3
3	BA	<0.8	<0.8
4	LC	1.4	<0.8
5	BA	<0.8	<0.8
6	BA	<0.8	<0.8
7	BA	<0.8	<0.8
8	FHF	<0.8	<0.8
9	BA	<0.8	<0.8
10	OTCD	<0.8	<0.8
11	BA	<0.8	<0.8
12	BA	<0.8	<0.8
13	BA	<0.8	<0.8
14	PSC	<0.8	<0.8
15	OTCD	<0.8	<0.8
16	BA	<0.8	<0.8
17	BA	<0.8	<0.8
18	BA	<0.8	<0.8
19	BA	<0.8	<0.8

OTCD ornithine transcarbamylase deficiency, *BA* biliary atresia, *LC* liver cirrhosis, *FHF* fulminant hepatic failure, *PSC* primary sclerosis cholangitis

Table 2 Data of the EA level by the EAA

Case	Original disease	Peripheral vein blood	Portal vein blood
1	OTCD	0.24	0.40
2	BA	0.22	0.31
3	BA	0.16	0.09
4	LC	0.16	0.42
5	BA	0.06	0.26
6	BA	0.01	0.38
7	BA	0.16	0.12
8	FHF	0.12	0.22
9	BA	0.36	0.23
10	OTCD	0.29	0.01
11	BA	0.08	0.16
12	BA	0.16	0.03
13	BA	0.03	0.24
14	PSC	0.24	0.02
15	OTCD	0.02	0.06
16	BA	0.26	0.15
17	BA	0.06	0.40
18	BA	0.13	0.32

OTCD ornithine transcarbamylase deficiency, *BA* biliary atresia, *LC* liver cirrhosis, *FHF* fulminant hepatic failure, *PSC* primary sclerosis cholangitis

In the beta-D glucan and NH_3 of the peripheral and the portal vein blood, the latter showed a significantly high level ($p = 0.001$ and $p < 0.001$, respectively) (Figs. 2, 3).

Discussion

The EAA is inferior to the LAL method that measures an absolute value in that it evaluates Et as a relative value. However, the EAA is capable of rapid and high-sensitive detection compared to the LAL method; therefore, in sepsis, which is a clinical state that requires prompt action, it plays an important role in diagnosis and treatment. In Europe and the USA, EAA is approved by the Food and Drug Administration and the efficacy thereof is recognized [8, 11]. Clinical states that cause high endotoxinemia mainly include infections with Gram-negative bacteria and bacterial translocation, and Et measurement is the mainstay in detecting sepsis. However, although the efficacy of EAA has been reported in the field of sepsis, it has been rarely reported in the other fields [9]. Et kinetics is affected by elevated levels of Et levels during intestinal mucosal damage, decreased liver function, and portosystemic shunt. Therefore, if the Et kinetics can be ascertained at the time of low endotoxinemia, it may become a useful index in fields other than the field of sepsis. Moreover, Et is processed by the hepatic reticuloendothelial system, and, therefore, there is a possibility that hepatic clearance may become a biological index for liver function [1, 2, 3]. It is believed that Et and cytokines in LT are major factors that influence circulatory dynamics and ischemia reperfusion injury [12], and Et kinetics during the perioperative period of LT can be a biological index that sensitively reflects changes in graft liver function. Furthermore, it has also been reported that through measurement of EA, rejection and infections can be differentiated or predicted [9]. It is believed that ascertaining Et kinetics post LT will be good news to the field of LT.

In this study, the EAA is superior to the LAL method in detection of Et during low endotoxinemia, because it ascertains Et kinetics. Moreover, it has been reported the LC group shows a significantly high beta-D glucan value because its clearance mechanism is impaired in the reticuloendothelial system in liver damage such as LC [3]. EA using EAA showed the hepatic clearance of beta-D glucan and NH_3, supporting the role of EAA in reflecting hepatic clearance and ascertaining Et kinetics.

If the Et level of the peripheral vein blood tends to elevate before LT, there is a possibility that the hepatic

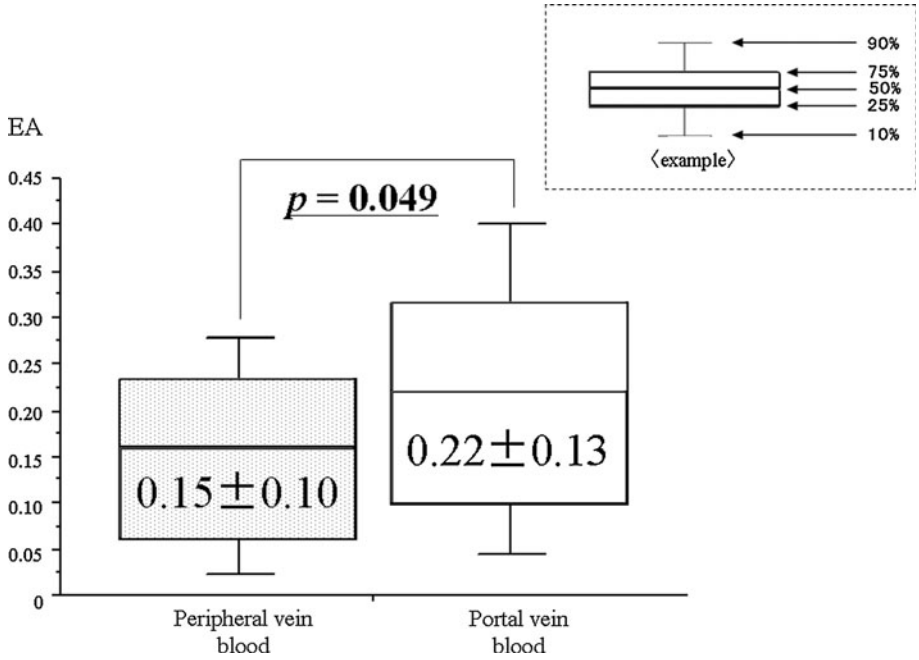

Fig. 1 *Box plots* showing the EA level distributions from the peripheral vein blood and the portal vein blood (EA levels at 10, 25, 50, 75, and 90%). The EA levels of the portal vein blood are significantly higher than of the peripheral vein blood ($p = 0.049$)

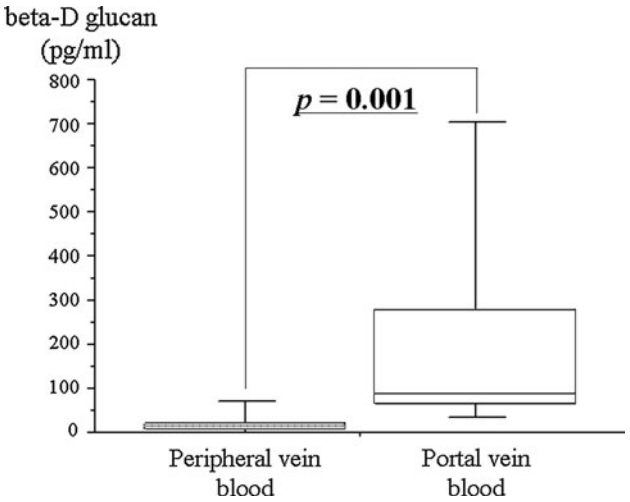

Fig. 2 *Box plots* showing the beta-D glucan level distributions from the peripheral vein blood and the portal vein blood (beta-D glucan levels at 10, 25, 50, 75, and 90%). The beta-D glucan levels of the portal vein blood are significantly higher than of the peripheral vein blood ($p = 0.001$)

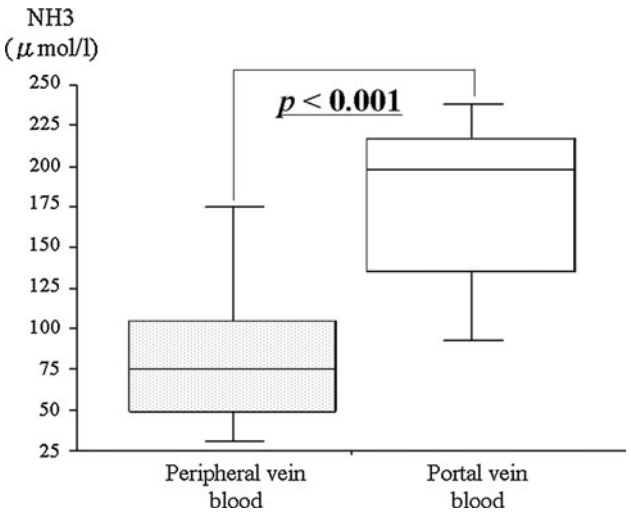

Fig. 3 *Box plots* showing the NH_3 level distributions from the peripheral vein blood and the portal vein blood (NH_3 levels at 10, 25, 50, 75, and 90%). The NH_3 levels of the portal vein blood are significantly higher than of the peripheral vein blood ($p < 0.001$)

reserve capacity is low or that lung damage may be easily caused via the portosystemic shunt. Therefore, in future the meaning of measurement by the EAA can be an index to determine the early indication of LT. Moreover, if Et level of the peripheral vein blood tends to increase following LT there is the possibility of low graft liver function due to rejection, infection, etc., thus leading to the early detection and early treatment of postoperative complications. Therefore, measuring the Et activity by EAA in LT may be suggested to be useful for evaluating the hepatic reserve capacity, graft liver function, and presence of infections. Both the further investigation and accumulation of cases in LT are, therefore, necessary hereafter.

Conclusions

The EAA is considered to be superior to the LAL method for the detection of Et, even in low endotoxinemia, and is also capable of elucidating the Et kinetics by accurately reflecting hepatic clearance.

Conflict of interest None.

References

1. Chan CC, Hwang SJ, Lee FY, et al. Prognostic value of plasma endotoxin levels in patients with cirrhosis. Scand J Gastroenterol 1997;32:942–946
2. Nakatani Y, Fukui H, Kitano H, et al. Endotoxin clearance and its relation to hepatic and renal disturbances in rats with liver cirrhosis. Liver 2001;21:64–70
3. Kimura Y, Nakao A, Tamura H, et al. Clinical and experimental studies of the limulus test after digestive surgery. Surg Today 1995;25:790–794
4. Elin RJ, Robinson RA, Levine AS, et al. Lack of clinical usefulness of the limulus test in the diagnosis of endotoxemia. N Engl J Med 1975;293:521–524
5. Bates DW, Parsonnet J, Ketchum PA, et al. Limulus amebocyte lysate assay for detection of endotoxin in patients with sepsis syndrome. AMCC Sepsis Project Working Group. Clin Infect Dis 1988;27:582–591
6. Romaschin AD, Harris DM, Riberio MB, et al. A rapid assay of endotoxin in whole blood using autologous neutrophil dependent chemiluminescence. J Immunol Methods 1998;212:169–185
7. Romaschin AD, Foster DM, Walker DM, et al. Let the cell speak: neutrophils as biologic markers of the inflammatory response. Sepsis 1998;2:119–125
8. Marshall JC, Foster D, Vincent JL, et al. Diagnostic and prognostic implications of endotoxemia in critical illness: results of the MEDIC study. J Infect Dis 2004;190:527–534
9. Hilmi I, Kellum JA, Planinsic R, et al. (2009) Endotoxemia is common following abdominal organ transplantation a d is associated with reperfusion and rejection. J Organ Dysfunction (in press).
10. Cohen J, McConnell JS. Observations on the measurement and evaluation of endotoxemia by a quantitative limulus lysate microassay. J Infect Dis 1984;150:916–924
11. Marshall JC, Walker PM, Foster DM, et al. Measurement of endotoxin activity in critically ill patients using whole blood neutrophil dependent chemiluminescence. Critical Care 2002; 6:342–348
12. Maring JK, Klompmarker IJ, Zwaviling JH, et al. Endotoxins and cytokines during LT: changes in plasma levels and effects on clinical outcome. Liver Transpl 2000;6:480–488