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Selective Plasma Exchange With Dialysis in Patients With Acute Liver Failure

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Abstract: Selective plasma exchange with dialysis is a blood purification therapy in which simple plasma exchange is performed using a selective membrane plasma separator while the dialysate flows out of the hollow fibers. To evaluate the effect of plasma exchange with dialysis, biochemical examination of the blood, for example, the oxidative stress regulation system and interleukin 18 levels, was performed in patients with acute liver failure. We studied four patients with acute liver failure in whom the therapy was performed (nine times in total). The degree of hepatic encephalopathy and interleukin 18 levels decreased significantly after treatment. However, total protein levels did not change significantly. The level of reactive oxygen species and total antioxidant capacity did not change significantly. Plasma exchange with dialysis may be a useful blood purification therapy in cases of acute liver failure in terms of the removal of water-soluble and albumin-bound toxins. **Key Words:** Acute liver failure, Oxidative stress regulation system, Plasma exchange with dialysis.

Plasma diafiltration (PDF) is a blood purification therapy in which simple plasma exchange (PE) is performed using a selective membrane plasma separator while the dialysate flows outside of the plasma separator; hemodiafiltration (HDF) is performed simultaneously (1–5).

Oxidative stress and the antioxidant defense systems have been studied in various liver diseases. Oxidative stress is considered to be associated with inflammation, fibrosis, and apoptosis of hepatic tissues (6–9). Oettl et al. reported that oxidative modification of circulating albumin leads to functional impairment, including alterations of binding properties; reversal of these oxidative modifications by extracorporeal liver support, for example, the molecular adsorbents recirculating system (MARS) and Prometheus treatments, could restore albumin function and reverse the mechanisms which otherwise would lead to multiple organ failure in acute-on-chronic liver failure (AoCLF) (10,11).

We have previously reported on the effectiveness of PDF in patients with acute liver failure (ALF) (4,5). We developed a new method of selective plasma exchange with dialysis (PED). The method is a blood purification therapy in which PE is performed using the Evacure EC-2A plasma separator while the dialysate flows outside the hollow fibers.

The aim of this study was to evaluate the effect of PED on biochemical parameters of blood such as the oxidative stress regulation system and IL-18 levels in patients with ALF.

PATIENTS AND METHODS

Subjects

The study was conducted with the informed consent of the patients and families involved, and with the approval of the ethics committee of the Akita University Hospital.

Four patients (one man and three women) with ALF were treated at the Akita University Hospital from May 2010 to August 2011. The causes of ALF were myelodysplastic syndrome (one case),

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TABLE 1. *Demographic data of patients with acute liver failure*

No.	Age (years)	Gender	Causes	MELD score	Total bilirubin (mg/dL)	INR	Creatinine (mg/dL)	Coma grade	No. of PED	Outcome
1	59	F	Myelodysplastic syndrome	40	25.6	2.03	0.67	V	2	Died
2	25	F	AML, GVHD	35	28.6	1.25	0.82	IV	2	Died
3	51	M	Guillain-Barre syndrome	31	2.5	2.03	2.68	IV	4	Survived
4	78	F	Fulminant hepatitis	40	12.2	2.55	1.36	II	1	Died

AML, acute myeloid leukemia; F, female; GVHD, graft-versus-host disease; INR, international normalized ratio; M, male; MELD, Model for End-Stage Liver Disease; PED, plasma exchange with dialysis.

graft-versus-host disease associated with acute myeloid leukemia (one case), Guillain-Barré syndrome (one case), and fulminant hepatitis (one case). The median age of the patients was 55 years (25–78 years). The median Model for End-Stage Liver Disease (MELD) score of the patients was 37.5 (31–40). The PDF treatment was carried out nine times in total. One patient survived and three died (Table 1).

In all groups, standard medical therapies focused on treating the precipitating events and the clinical problems associated with liver failure. Sources of bleeding were identified and treated. When indicated, proton pump inhibitors or H_2-blockers were given. Infections were assessed and treated with appropriate antibiotics. Patients with renal failure requiring dialysis received intermittent or continuous (if hemodynamically unstable) HD if they developed anuria, life-threatening electrolyte disorders, or uremia.

Fulminant hepatitis was defined as hepatic failure that developed in a previously healthy individual within 8 weeks of the onset of liver disease, a grade II or higher coma, and a prothrombin time (PT) of less than 40% of the normal or international normalized ratio of more than 1.5.

Acute liver failure was defined as acute, severe liver dysfunction (except for the case of fulminant hepatitis), with a serum total bilirubin (T-Bil) level higher than 5 mg/dL, a PT of less than 40% of the normal or international normalized ratio of more than 1.5, and an ensuing grade II or higher coma.

Procedures

Blood access was established through a double-lumen catheter via the patient's jugular, subclavian, or femoral vein. In this study, we used the Evacure EC-2A plasma separator (Kawasumi Laboratories, Tokyo, Japan). The PED session lasted 8 h and the blood flow rate was 100 mL/min. Dialysate fluid (Na^+ 140.0 mEq/L, K^+ 2.0 mEq/L, Ca^{2+} 3.5 mEq/L, Mg^{2+} 1.0 mEq/L, Cl^- 111.0 mEq/L, CH_3COO^- 3.5 mEq/L, HCO_3^- 35.0 mEq/L, $C_6H_{12}O_6$ 100 mg/dL; Sublood-BS, Fuso Pharmaceutical, Osaka, Japan) was infused at a dialysate flow rate of 2000 mL/h. We infused 1800 mL of normal fresh-frozen plasma (FFP; Japanese Red Cross) at a replacement flow rate of 230 mL/h. Nafamostat mesilate was administered at an initial dose of 30 mg/h and adjusted to maintain an activated coagulation time of 150 to 180 s (Fig. 1).

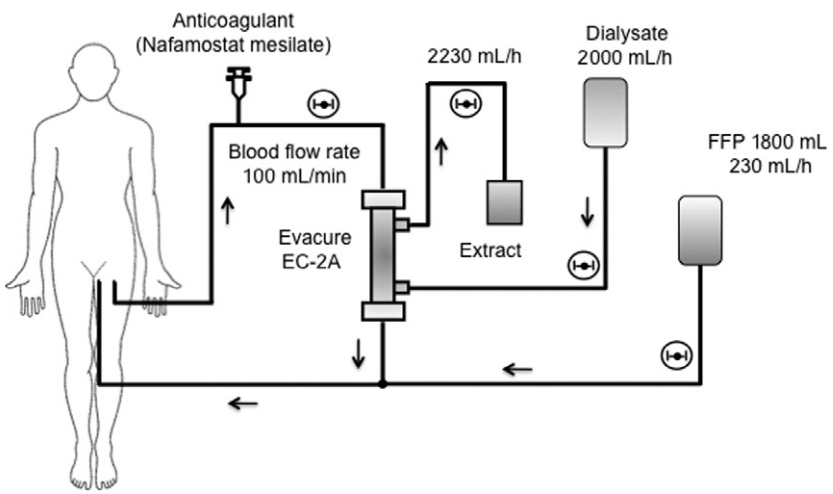

FIG. 1. Schematic representation of the flow of selective plasma exchange with dialysis (PED). FFP, fresh-frozen plasma.

Study observations

The grading of hepatic encephalopathy was based on the criteria established at Inuyama Symposium in 1982 (12). Blood samples for assays of T-Bil, PT (or international normalized ratio), IL-18, reactive oxygen species, and total antioxidant capacity were collected in endotoxin-free heparinized blood-specimen tubes at the start and immediately after each plasmapheresis session. The samples were immediately centrifuged at 1000× *g* for 5 min and stored at –80°C until being assayed.

Interleukin-18 levels were determined using an ELISA (MBL, Nagoya, Japan); the detection limit was 12.5 pg/mL (normal, 126 ± 44.5 pg/mL).

Reactive oxygen species in the serum were evaluated by using the Diacron reactive oxygen metabolites (d-ROMs) test (Wismerll, Tokyo, Japan) (13,14). The test is based on the concept that the amount of organic hydroperoxides present in serum is related to the free radicals from which they are formed. When the serum sample is dissolved in an acidic buffer, the hydroperoxides react with the transition metal ions liberated from the proteins in the acidic medium and are converted to alkoxy and peroxy radicals. These newly formed radicals are able to oxidize an additive (*N*, *N*′-diethyl-*p*-phenylenediamine) to the corresponding radical cation. The concentration of this persistent species can be easily determined through spectrophotometric procedures (absorption at 505 nm). The normal values of the test are between 250 and 300 U CARR (Carratelli units), where 1 U CARR corresponds to 0.8 mg/L hydrogen peroxide. Values outside this range are considered indicative of an alteration in the equilibrium between the pro- and antioxidant capability of subjects. Values of more than 300 U CARR indicate a condition of oxidative stress.

The total antioxidant capacity in serum was evaluated manually by using the OXY-adsorbent test (Wismerll, Tokyo, Japan), according to a fixed-time analysis (14,15). This test is based on the capacity of a massive dose of hypochlorous acid (HClO) to oxidize the physiologic antioxidant reef (uric acid, glutathione [GSH], thiol groups, vitamins, GSH peroxidase [GSH-Px], superoxide dismutase [SOD], catalase, etc.). The efficacy of the antioxidant system can be monitored indirectly by measuring the excess of HClO in serum. As HClO reacts with a correctly buffered chromogenic substrate, a colored complex is formed, which can be measured photometrically, presenting a maximum peak of absorbance at 505 or 546 nm. The concentration of the colored complex is directly proportional to the concentration of HClO and indirectly proportional to the antioxidant capacity. The normal value of the test is >350 μmol/mL HClO.

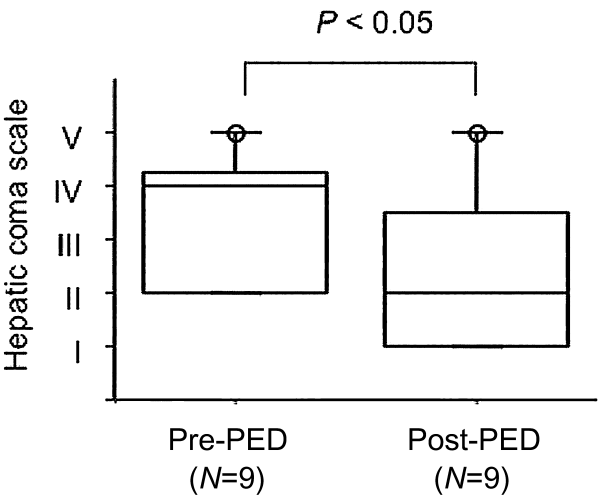

FIG. 2. Degree of hepatic encephalopathy before and after plasma exchange with dialysis (PED).

Data analysis

Data for the parameters are expressed as median values with their ranges. Differences were evaluated for significance by using Wilcoxon's signed rank test. A *P*-value of less than 0.05 was considered significant.

RESULTS

All procedures were performed smoothly and safely even on critically ill patients.

The changes in the coma grade are shown in Figure 2. The grade decreased significantly after treatment (pretreatment IV [II–V] vs posttreatment II [I–V], $P = 0.0196$). The changes in total protein and IL-18 levels are shown in Figure 3. No significant difference was observed in total protein levels before and after treatment (pretreatment 5.3 g/dL [3.6–4.2 g/dL] vs posttreatment 4.85 g/dL [4.2–6.3 g/dL], $P > 0.9999$). The IL-18 levels decreased significantly after treatment (pretreatment 671.0 pg/dL [328.0–1540.0 pg/dL] vs posttreatment 548.0 pg/dL [290.0–810.0 pg/dL], $P < 0.0077$). The changes in the oxidative stress regulation system are shown in Figure 4. No significant difference was observed in the results of the d-ROMs test before and after treatment (pretreatment 148.0 U CARR [61.0–249.0 U CARR] vs posttreatment 166.0 U CARR [104.0–230.0 U.CARR.], $P = 0.1386$). No significant difference was observed in the results of the OXY-adsorbent test before and after treatment (pretreatment 357.6 μmol/mL HClO [280.1–439.0 μmol/mL HClO] vs posttreatment

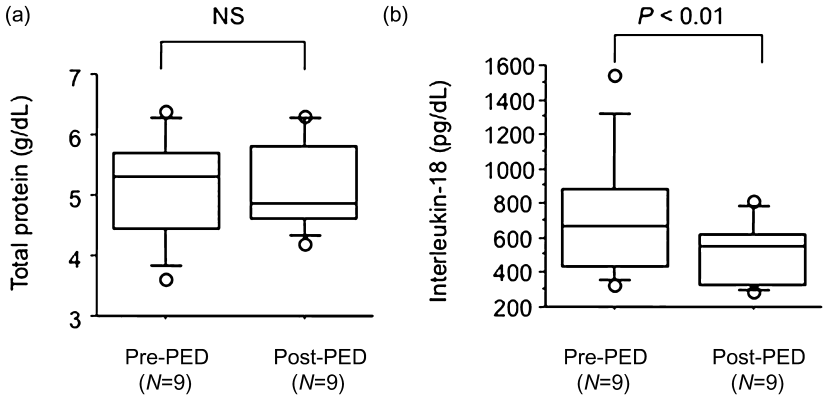

FIG. 3. Serum concentrations of total protein and interleukin (IL)-18 before and after plasma exchange with dialysis (PED). NS, not significant. (a) Total protein. (b) Interleukin 18. NS, not significant; PED, plasma exchange with dialysis.

386.4 μmol/mL HClO [287.5–427.0 μmol/mL HClO], $P = 1731$).

DISCUSSION

The Evacure EC-2A plasma separator used for PED in this study has a pore size of 0.01 μm, which is much smaller than that of the conventional plasma separation membrane (0.2 to 0.4 μm). This membrane has a sieving coefficient of 0.3 for albumin and, thus, can selectively remove low- or intermediate-molecular-weight albumin-bound substances. In addition, coagulation factors are preserved because this membrane has a sieving coefficient of 0 for fibrinogen and immunoglobulin M (9).

With PE, it is difficult to completely control increases in the citrate concentration even when continuous hemodiafiltration (CHDF) is performed concomitantly with the administration of large amounts of FFP (3200 to 4000 mL) (16,17). On the other hand, with PED, the increase in the citrate concentration is inhibited more effectively because smaller amounts of FFP (1800 mL) are required and because HD is performed simultaneously.

Interleukin-18 is a protein with a molecular weight of 18.3 kDa and is produced by activated macrophages, particularly by Kupffer cells in the liver (18). We have reported that IL-18 affects patient morbidity in ALF by mediating the excess production of inflammatory cytokines, including TNF-α (16,19,20). In short, IL-18 is considered to be involved in the pathophysiology of ALF. Li et al. reported that PDF could effectively remove almost all inflammatory mediators in an in vitro sepsis model (21). In our previous PDF studies, excessive amounts of both water-soluble and albumin-bound substances such as cystatin C, T-Bil, IL-6, and IL-18 were reduced after treatment (1–5). In this study, IL-18 levels decreased significantly with treatment. Hepatic encephalopathy develops following massive liver necrosis as a consequence of increased plasma ammonia levels due to dysfunction of the urea cycle in hepatocytes. Encephalopathy was improved significantly with treatment. Thus, the PED technology may remove both water-soluble and albumin-bound toxins.

Oxidative stress has been postulated as a key mechanism in the pathogenesis of ALF (6–10). Oettl et al. reported that the redox state of albumin serves as a marker for oxidative protein modification and AoCLF patients have been found to be the group with the highest levels of human non-mercaptalbumin-2, which indicates the particular

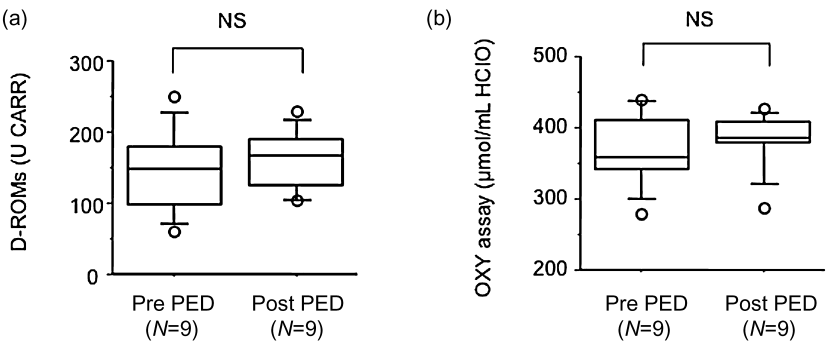

FIG. 4. Oxidative stress regulation system before and after plasma exchange with dialysis (PED). NS, not significant. (a) Derivatives of reactive oxygen metabolites. (b) Total antioxidant capacity. D-ROMs; Diacron reactive oxygen metabolites; HClO, hypochlorous acid; NS, not significant; OXY assay, total antioxidant capacity; PED, plasma exchange with dialysis; U CARR, Carratelli units.

importance of oxidative stress in liver disease (10). Furthermore, both MARS and Prometheus treatments lead to transient improvements of the redox state of albumin, which could be beneficial in the treatment of AoCLF (11).

On the contrary, the concentrations of reactive oxygen species showed lower than normal values before PED and increased after treatment. The values of the total antioxidant capacity were lower than the normal range before PED and increased after treatment, too. The oxidative stress regulation system may be suppressed in severe ALF. However, this mechanism is still unclear and further investigations need to be performed. PED could also be beneficial in the treatment of ALF in terms of the control of the oxidative stress regulation system.

Compared to PDF treatment, PED should be performed especially in the following two conditions: severe hypoproteinemia and severe thrombopenia. In PDF, the plasma separation membrane has a sieving coefficient of 0.3 for albumin and HDF is performed. In our previous study, the levels of total protein increased after treatment and those of albumin did not change significantly in PDF. Therefore, it may be safe to perform PDF in the condition of hypoproteinemia. On the other hand, HD is performed in PED. PED is considered to be safer than PDF. For the case of severe thrombopenia, HF may have a mechanical and hemolytic effect in PDF. Therefore, PED is considered to be safer than PDF.

The number of times PED was performed in the surviving patients was greater than that in the non-surviving patients. No adverse events were observed during PED. The MELD score in the non-surviving patients tended to be higher than that in the surviving patients, which indicated that the severity of ALF in the non-surviving patients was too high to respond to PED. Therefore, the doctor in charge and the family of the patient decided to discontinue aggressive treatment such as PED after performing it once or twice. The inclusion criteria for PED should be redefined to ensure that the procedure can be started at an early stage of ALF.

CONCLUSIONS

Plasma exchange with dialysis is a simpler modality than plasma diafiltration and it may be a useful artificial liver support system for patients with acute liver failure in terms of medical economics and the removal of water-soluble and albumin-bound toxins. Further controlled clinical trials with survival as an endpoint are in progress and will help determine the efficacy and safety in patients with ALF.

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