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Development of Reliable Artificial Liver Support (ALS)–Plasma Exchange in Combination with Hemodiafiltration Using High-Performance Membranes

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A new artificial liver support system (ALSS) consisting of plasma exchange (PE) in combination with hemodiafiltration (HDF) using high-performance membranes of polymethyl metacrylate (PMMA) and cellulose triacetate (CTA) was developed to efficiently remove middle molecules from plasma and treat fulminant hepatic failure (FHF) complicated by the onset of hepatic coma. Twenty-seven patients with FHF due to viral hepatitis, two with type A (HA), nine with type B (HB), and 16 with type non-A, non-B (NANB) underwent therapy with this new ALSS over the last five years. Three patients with an exacerbation of chronic HB and 15/16 with type NANB hepatitis were treated with interferon (IFN) also. Of these, 25 patients (92.6%) regained consciousness and 15 (55.6%) [1/2 (50%) with type A, 6/9 (66.7%) with type B and 8/16 (50%) with type NANB hepatitis] survived. Including four patients who survived with intensive care and plasma exchange alone, 19/31 (61.3%) patients survived. Because of its biocompatibility, both survivors and nonsurvivors could be sustained with the ALSS without complications for long periods (19.3 days for the survivors and 32.4 days for nonsurvivors). With this ALSS the ability to sustain life for such prolonged periods allows hepatic regeneration to occur and result in patient survival. It is anticipated that this new ALSS will not only be of value in cases of fulminant hepatic failure but that it may also play a role in sustaining life for those awaiting liver transplantation.

KEY WORDS: artificial liver support system; plasma exchange; hemodiafiltration.

Fulminant hepatic failure (FHF) remains one of the life-threatening diseases for which adequate medical therapy does not exist. Several artificial liver support systems (ALSS) have been devised and have been applied to the treatment of FHF. The ALSS tested in substantial numbers of FHF patients include exchange transfusion (ET) (1), plasma exchange (PE) (2), charcoal hemoperfusion (HP) (3), charcoal plasma perfusion (PP) (4), and hemodiafiltration (HDF) using a polyacrylonitrile (PAN) membrane (5).

The effect of ET on survival of FHF patients was not demonstrated by a controlled trial (6). Although charcoal HP was at one time claimed to have achieved a high survival rate (10/22) in an open study (3), its effectiveness could not be confirmed by a subsequent large scale controlled trial (7). The survival rate with PAN-HDF has been reported at 21.5% in one report (5), and as being 33% in another

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(8). Due to the relatively poor therapeutic effectiveness of these ALSS, liver transplantation currently is advocated as the best treatment for FHF (9, 10).

Unfortunately, not all patients with FHF can be treated with liver transplantation because compatible donor livers are not readily obtainable (11). Worse yet, liver transplant patients, particularly those with viral liver diseases, frequently experience recurrent viral liver disease, which can on occasion present as FHF (12).

The liver plays a central role in the metabolism of various materials, including carbohydrates, proteins, amino acids, lipids, vitamins, and hormones. From a clinical viewpoint, however, two functions are particularly important. These are: (1) The production of plasma proteins, especially coagulation factors; and (2) the metabolism of various molecules having a potential for central nervous system (CNS) toxicity. Loss of these two functions is responsible for the two major clinical problems associated with FHF: bleeding and coma. Thus restoration of these two hepatic functions is a prerequisite for an effective ALSS. The poor effect of all existing ALSS can be attributed to a failure to either completely or at least partially restore these two functions.

In Japan PE is currently the most frequently used method of liver support in cases of FHF because of the technical advances in the practice of PE and the readily available supply of fresh frozen plasma (FFP) provided by the Japanese Red Cross. PE serves the two critically important hepatic functions required of an effective ALSS by supplying plasma components that are synthesized by the liver and removing toxic materials that are normally metabolized by the liver. Experience has shown that although PE is effective in restoring plasma components, it has only a limited value in removing putative neurotoxic metabolites from a large body pool (13). As a result, most patients with severe FHF die of coma despite aggressive PE.

With the aim of developing a more reliable ALSS, we have combined PE with a method capable of efficient and safe removal of putative neurotoxic substances thought to be responsible for hepatic coma. We have developed HDF using a recently developed high-performance membrane with large pores, which allows proteins to be filtered for the effective removal of middle-sized molecules (MMs), which have been thought to be responsible for hepatic coma (14). Preliminary use of this new ALSS has shown it to be effective in reversing grade V hepatic coma in a patient with fulminant liver failure (15). In the present communication, the experience in a substantially larger number of patients with viral-induced FHF treated with ALSS is reported.

MATERIALS AND METHODS

The subjects in the present study were 27 consecutive patients with FHF including six with late-onset hepatic failure (LOHF), with coma occurring 8–24 weeks after the onset of hepatitis (16). All were treated at Showa University Fujigaoka Hospital in the last five years. In each case their coma had not resolved with standard intensive care.

Virological Studies. The methods utilized for the determination of various hepatitis markers were as follows: IgM HA-Ab, HB_sAg, and IgM HBc Ab were determined by RIA; anti-HCV (C-100-3) was measured using a HCV-Ab ELISA kit (Ortho Diagnostic Systems); HBV DNA polymerase (DNA-P) was assayed; HBV DNA was detected using the polymerase chain reaction (PCR), using primers capable of amplifying nucleotide (nt) sequences 1653–1972; HCV RNA was assayed by a two-stage PCR using two pairs of oligonucleotides from the 5′-noncoding region of HCV as primers (17); anti-core for HCV was determined by ELISA using two core peptides (CP9 and CP10) (18, 19).

In order to avoid false positive results for the various HCV markers due to contamination by blood donor plasma, all virus markers were assayed prior to the start of the plasma exchange (PE) therapy.

Type A hepatitis was diagnosed by the identification of IgM HA-Ab. Acute type B hepatitis was diagnosed by the presence of a positive HB_sAg and/or IgM HBc-Ab. Two patients were negative for HB_sAg but had IgM HB_c and were diagnosed as having type B hepatitis. Both were positive for HBV DNA by PCR. An acute exacerbation of HB in an HBV carrier was diagnosed by a history of known HB_sAg positivity for more than six months, the presence of HBV DNA and HBV DNA-P, and a markedly elevated IgG HB_c level. Type C hepatitis was diagnosed by the presence of either a positive anti-C-100, HCV RNA or HCV anti-core result.

Morphological and Histological Diagnosis. All patients underwent ultrasonographic (US) and computer tomographic (CT) examinations for determination of liver volume. US was performed on admission and at least once a week thereafter. CT scanning was performed on admission and at least twice a week thereafter. Laparoscopy was performed in 12 of the 15 patients. Liver biopsy was obtained in 11 of the 12 patients who underwent laparoscopic examinations. Three serial biopsies (**5, 4 & 3**) were obtained in three patients. Four patients had two biopsies. An autopsy was obtained in 10 of the 12 patients that died.

ALSS. The high-performance membranes used were a polymethyl metacrylate membrane (PMMA, BK series: Toray Medical Inc., Tokyo) and a cellulose triacetate membrane (CTA, Nipro Co., Tokyo), which were originally developed for the removal of α_2-microglobulin (mol wt 118.00) (20). According to the manufacturer, the PMMA and the CTA membranes had an inulin (mol wt 5200) permeability three times greater than the PAN

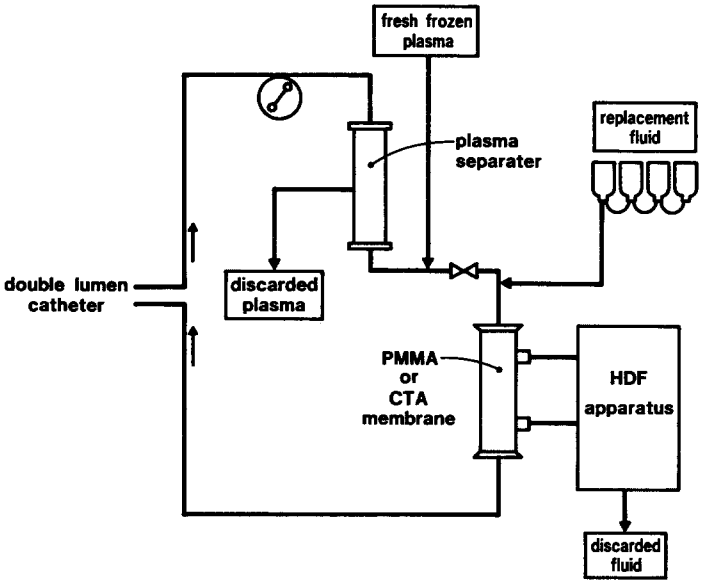

Fig 1. Circuit diagrams for the new artificial liver support system (ALSS).

membrane used by others. Before its clinical use, the biocompatibility of the PMMA membrane was assessed in dogs (21). The biocompatibility of the CTA membrane was assessed directly with its clinical use. Adverse effects, including hemolysis, significant platelet loss, and DIC attributable to either the PMMA HDF or CTA HDF, were not observed.

Blood access was established with a double-lumen catheter inserted into a central vein. Two patients who required continuous ALSS for more than three months had a surgical A-V fistula created using the dorsalis pedis artery. A 10-mg bolus of nafamostat mesilate, a proteinase inhibitor (Futhan, Torii Pharmaceutical Co., Tokyo) was added to the ALSS circuit and was delivered at the rate of 20 mg/hr in the course of the ALSS treatment just prior to the initiation of PE.

PE was performed using a membrane separation method marketed as Plasmaflo (Asahi Medical Co., Tokyo). FFP was supplied by the Japanese Red Cross Kawasaki Blood Center (Kawasaki City, Kanagawa Prefecture). The amount of FFP used during PE was adjusted to keep the patient's PT level above 30% and averaged 3.5 liters. Blood, the plasma of which was exchanged as FFP, was drawn into the circuit of the PMMA or CTA HDF. HDF was performed using a commercially available HDF apparatus (TR 701: Toray Medical Inc., Tokyo). Filtration was performed at a flow rate of 4–6 liters/hr using a bicarbonate buffer, pH 7.4, having a potassium concentration of 4.0 meq/liter. The volume of the substitution fluid was adjusted over a range of 6–30 liters, depending on the patient's response. Dialysis was performed concomitantly at a flow rate of 500 ml/min using a conventional acetate buffer. The circuit diagrams for the ALSS are shown in Figure 1. All three patients with an acute exacerbation of HBV and 11 of the 15 patients with NANB FHF were treated with IFN-β daily at a dose of 300 MU for 3–60 days.

RESULTS

Two patients were diagnosed as having type A hepatitis. Six patients had acute type B hepatitis. Three patients had an acute exacerbation of their chronic HB carrier state. All three developed FHF following repeated intensive chemotherapy for non-Hodgkin's lymphoma in spite of being negative for HB_eAg and having completely normal liver function tests before the start of their chemotherapy. The remaining 15 patients were diagnosed as having non-A, non-B hepatitis because of negative tests for all HAV and HBV markers together with negative tests for other viruses known to cause hepatitis and negative results when tested for the presence of various autoantibodies.

Three NANB patients were positive for all three HCV markers assessed. Four NANB patients were positive for two of the HCV markers utilized and six were positive for only one HCV marker. Thus, 13 of 16 NANB patients were diagnosed as having type C hepatitis. Both patients with type A hepatitis were positive for one or another HCV marker. This high rate of superinfection in type B hepatitis is explained by the fact that all six had a history of prior blood transfusion. Thus, 21/27 (77.8%) had evidence for recent or past HCV infection. The cause of the FHF in the HAV group was assessed to the HAV because the IgM HA level was elevated throughout the course of the liver failure. The cause of the FHF appeared to be HBV in four patients

because their liver failure resolved after the cessation of identifiable HBV replication. In one patient, however, liver failure was protracted after the loss of HB_sAg. HCV infection may have played a role in the hepatic failure in this particular case.

Fifty-five out of 27 patients (92.6%) regained consciousness, and 15 patients (one patient with type A, six patients with type B, and eight patients with type NANB) survived. Twelve patients (one patient with type A, three patients type B, and eight patients type NANB) died. The survival rate was 1/2 (50%) for type A, 6/9 (67%) for hepatitis B, and 8/16 (50%) for hepatitis NANB. Another two HB patients with FH, one with hepatitis A + C and one with fatty liver of pregnancy survived with PE alone. Overall, 19 of 31 patients (61.3%) with FHF treated at this institution survived.

The 15 surviving patients received 3–75 ALSS sessions (average 16.1 sessions) over a mean of 19.3 days. The 12 patients who died were given 4–115 sessions (average 16.1 sessions over a period of 32.4 days). The volume of FFP utilized for one session of PE was 2.4–4.8 liters (3.2 ± 0.61 liters) for the surviving patients and 3.2–4.8 liters (average 3.5 ± 0.6 liters) for those who died. The volume of replacement fluid utilized in one session of HDF was 6–20 liters (average 15.3 ± 6.4 liters) for the surviving patients and 14–30 liters (average 23.6 ± 6.1 liters) ($P < 0.01$) for those who died. Thus HDF was utilized more intensively in the latter cases but to no avail.

The ALSS was initiated at grade I coma in two, at grade II coma in six, and at grade III coma in seven of the 15 surviving patients. It was started at grade II coma in 10, at grade III coma in one, and at grade IV coma in one of the 12 patients who died. No significant difference in coma grade between survivors and nonsurvivors was evident at the time the ALSS was started.

The 27 patients included in this study could be divided into three groups (group I: survivors; group II: patients who died of either a complication or interruption of the ALSS; and group III: patients who died of coma despite ALSS). Several parameters reflecting residual hepatic function in each of these three groups were compared including minimum prothrombin time (PT), maximum total bilirubin (TBil), ratio of direct bilirubin to total bilirubin (DBil/TBil) and minimum BUN level. The minimum PT level was 22.6 ± 11.3%, 16.3 ± 14.7%, and 14.0 ± 12.3 min in groups I, II, and III, respectively. Because of the wide variability in each group, these values did not differ between groups. The maximum TBil level was 17.7 ± 9.2, 26.2 ± 3.4 and 24.8 mg/dl, respectively, for groups I–III; again no group differences were evident. The minimum DBil/TBil was 0.58 ± 0.12, 0.49 ± 0.17, and 0.19 ± 0.14, respectively, for groups I–III. This parameter statistically distinguished group I from group III ($P < 0.01$) and group II from group III ($P < 0.02$). The minimum BUN level was 10.3 ± 3.9, 10.3 ± 6.6 and 2.7 ± 3.9 mg/dl for groups I–III, respectively. This value distinguished group I from group III ($P < 0.001$) and group II from group III ($P < 0.01$). The hepatic volume measured by abdominal CT was 507–1427 ml (average 895 ± 412 ml) for 14 of the 15 patients in group I. Liver weight at autopsy ranged from 450 to 1040 g (average 688 ± 218 g) in four of the five patients in group II who died. The liver volume of the one patient who was not autopsied was 356 ml by CT scan. Liver weight at autopsy was 210–790 g (average 495 ± 227 g) in six of the seven patients in group III who died. The liver volume of the one patient who was not autopsied in this group was 585 ml by CT at the time of death.

The complications of hepatic failure experienced in this series, in addition to coma, included pulmonary aspergillosis, sepsis, adult respiratory distress syndrome (ARDS), and bleeding from esophageal varices. Each of these caused a patient's death. Nonvariceal gastrointestinal bleeding occurred in one surviving and six nonsurviving patients. Renal failure occurred in one surviving patient and two patients who died. Brain edema detected by CT was noted in two surviving patients and in three patients who died. In each case, it occurred during grade IV or V coma. Fungal and bacterial infection and ARDS occurred in seven surviving patients and in one patient who died.

In the following case report, the course of ALSS treatment of a 30-year-old female who had the lowest (worst case) DBil/TBil, BUN, and liver weight values in this series is described. The patient maintained consciousness while ingesting a 60-g protein 1600-cal diet with the use of ALSS requiring 51 liters of FFP and 30 liters of replacement fluid.

Her illness had begun with nausea and dark urine on February 17, 1989. She was noted to have an AST level of 1468 IU/liter, ALT of 1199 IU/liter, TBil 11.3 mg/dl, and DBil 5.2 mg/dl on March 6, and was hospitalized at a nearby hospital. On March 25, she was referred to us for ALSS. All HA and HB markers were negative. After admission her TBil rose to 33.6 mg/dl with a DBil level of 12.4 mg/dl. She had grade II coma on March 21 and began PE

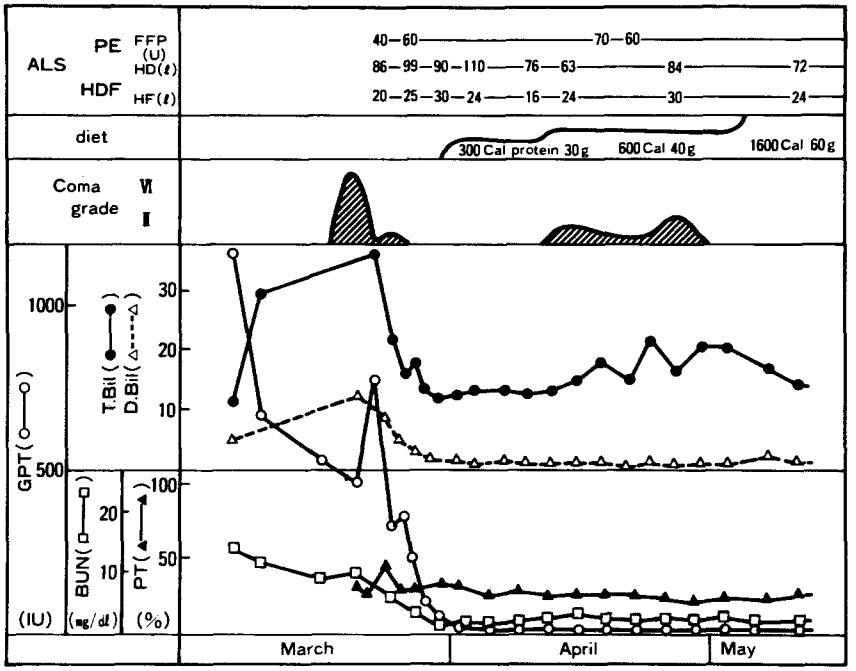

Fig 2. Clinical course of a 30-year-old female with type C FHF whose liver weight at autopsy was only 210 g. Note the marked reduction of direct bilirubin (DBil) as compared to the total bilirubin (TBil) and the BUN. The patient tolerated a 60-g protein 1600-cal diet while undergoing ALSS.

using 4.8 liters of FFP. Because her coma progressed to grade V despite PE, CTA HDF using 20 liters of bicarbonate buffer was performed. She became responsive after two runs of the ALSS and was transferred to us for additional ALSS treatment on March 25.

On admission, physical examination revealed profound jaundice, anemia, and grade II coma. Liver dullness was only 6 cm to percussion. The results of her laboratory tests on admission demonstrated an abnormally low direct bilirubin level compared with the level of the total bilirubin and a very low BUN level. Her PT was low at 29.3% despite two runs of PE on each of the preceding two days. Fischer's ratio (plasma Val + Leu + Ile/Try + Phe) was low at 0.57 (normal range 3.0–4.0), and her plasma Gln was high at 2414 nmol/ml (normal range 478.3–658.3 nmol/ml).

After three additional runs of PE using 4.8 liters of FFP and CTA HDF using 30 liters of fluid, she regained consciousness. Although her PT transiently rose to around 60% after PE, it regularly fell to levels of 20–25% just prior to the start of the next PE. Any reduction in the amount of substitution fluid to a value less than 24 liters invariably induced coma. Her DBil/TBil ratio fell to its lowest value of 0.05 on the 36th hospital day (DBil 1.0 mg/dl and TBil 20.1 mg/dl). Her BUN level fell to its lowest value 0.6 mg/dl on the 14th hospital day (Figure 2).

Because consciousness could be maintained by daily PE using 4.8 liters of FFP and CTA HDF utilizing 30 liters of the substitution fluid, oral nutrition was started with 300 cal of carbohydrate beginning on the 6th hospital day. Protein was added to the diet on the 10th hospital day with an increase in the caloric intake to 800 cal. She was finally placed on a 60-g protein, 1600-cal diet without any signs of encephalopathy. Moreover, she occasionally enjoyed overnight stays at home between ALSS sessions which lasted about 10 hr. She regularly experienced severe hypoglycemic episodes in the early morning hours. Serial abdominal CT obtained every other week showed progressive shrinkage of her liver (Figure 3). The calculated Fischer's ratio fell as low as 0.22 and the plasma Gln level often exceeded 10,000 nmol/ml after the initiation of the oral diet.

After the 100th hospital day she began to lapse into coma again despite continuation of the ALSS, and ultimately she died of deepening coma on the 115th hospital day. At autopsy, her liver weight was only 210 g. Histologic examination revealed only a

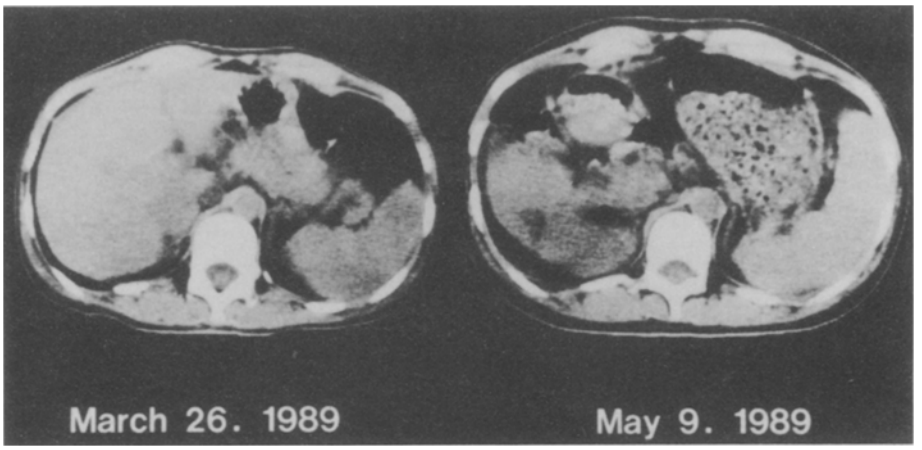

Fig 3. Abdominal CT findings of the patient in group III on March 26 and May 9, 1989. Note the progression of the liver atrophy during this interval.

few residual liver parenchymal cells forming pseudo-bile ductules around the portal areas (Figure 4).

DISCUSSION

A new ALSS system consisting of PE and HDF using high-performance membranes [polymethyl metacrylate (PMMA) and cellulose triacetate (CTA)] was devised to enhance the removal of the middle-sized molecules thought to be responsible for hepatic coma. Before its use clinically, the biocompatibility of the PMMA membrane was assessed using dogs with experimental FHF, because previous experience with PP using uncoated charcoal had shown that, although charcoal PP is effective in relieving coma in patients with mild liver failure, it causes severe bleeding due to thrombocytopenia. Subsequent experimental studies using dogs with experimental FHF disclosed that the charcoal PP enhanced coagulation and fibrinolysis resulting in severe DIC (22). Activation of factor XII on the surface of uncoated activated charcoal may be responsible for this untoward phenomenon.

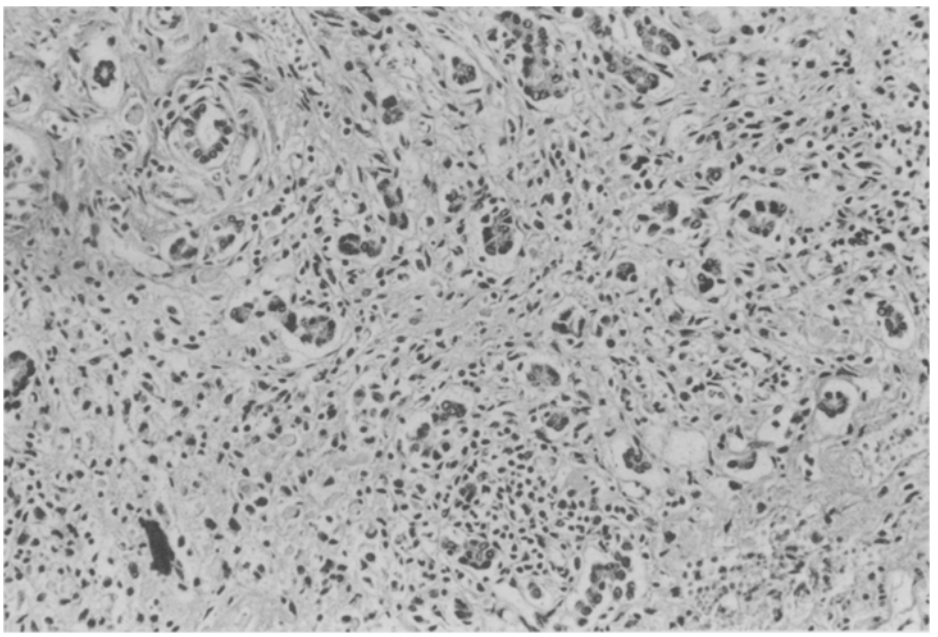

Fig 4. Autopsy finding of the liver. Massive hepatic necrosis with barely visible liver parenchymal cells forming pseudo-bile ductules.

Unlike charcoal, the PMMA membrane used in this system is biocompatible and both the PMMA and the CTA membrane produced no adverse clinical effects.

The effectiveness of this new ALSS is demonstrated by the observation that 25/27 (92.6%) patients with FHF treated with it completely recovered consciousness from grade I–V coma. Six of these 23 patients lapsed into coma again as hepatic regeneration failed to occur. Nonetheless, this high rate of transient as well as complete recovery from hepatic coma is in marked contrast with the 50% value reported for charcoal HP (3), 17/41 (43.6%) (5), and 9/24 (37.5%) (8) for the PAN HDF. Moreover, it is considerably better than the 14/26 (53.8%) recovery reported by us using charcoal PP (4). The four patients who failed to respond to the ALSS and the six patients who relapsed into coma all had either markedly atrophied livers on admission or experienced progressive hepatic atrophy despite ALSS. This point is documented by the average liver weight of seven autopsied cases, which was 460 ± 170 g. The lowest mean serum DBil/TBil ratio that reflects hepatic bilirubin conjugation capacity was 0.19 ± 0.14 for the seven patients who died. Their mean lowest BUN level, which reflects overall urea cycle capacity of the liver, was markedly reduced at 2.7 ± 3.9 mg/dl. Moreover the extremely high plasma Gln level, the end product of an alternative ammonia detoxifying pathway, further documents the severity of the urea cycle dysfunction in these seven cases. It is noteworthy that even the most severe case, in which liver weight was only 210 g at autopsy and in which occurred the lowest DBil/TBil ratio and BUN levels at 0.05 and 0.6 mg/dl, respectively, could be maintained conscious while ingesting a 60-g protein 1600-cal diet while on the ALSS and requiring 4.8 liters of FFP and 30 liters of substitution fluid.

Most importantly, the effectiveness of this ALSS was demonstrated by the high rate (15/27, 55.6%) of survival among patients who received the therapy. This survival rate has to be compared with that reported for ET (0/8, 0%) (6), for charcoal HP started at grade IV coma (10/22, 45.5%) (3), for PAN HDF in two reports (9/42, 22.0% (5) and 8/24, 33.3% (8)), and for charcoal PP in our own hands (5/26, 19.2%) (4).

In the present study the ALSS was instituted at a coma grade of less than III in 26 of the 27 patients. The early application of ALSS may have contributed to the high survival rate (23). It needs to be recalled, however, that the major determinant of prognosis in cases of FHF is the etiology of FHF. Thus, with charcoal HP while 54.7% and 63.6% of patients in grade III coma due to acetaminophen overdose or hepatitis A and B survive, only 12.5% and 33.3% of patients with NANB and halotheme hepatitis, respectively, survive (24).

Virological study of the 27 patients in this study disclosed that two had type A, nine had type B, and 16 had NANB hepatitis. Evidence for HCV infection was present in 13 of 16 patients with NANB, both of the type A hepatitis cases, and six of nine with hepatitis B also had evidence of prior HCV infection. Thus, the HCV infection rate in this series was 21/27 (77.8%). The high rate of coinfection in the cases with hepatitis A was unexpected. The high prevalence of HCV in cases of hepatitis B is explained by the fact that all of them had received blood transfusion before the onset of their FHF.

Currently it is not known how HCV infection influences the clinical course of either hepatitis A or B associated FH. The two cases with hepatitis A in the present series experienced unusually protracted liver failure. One of these, although manifesting the clinical features of acute hepatitis at the onset, ultimately died of bleeding of an esophageal varix with a hepatic histology consistent with rapidly progressive cirrhosis. Another patient with hepatitis B failed to recover despite clearance of HB_sAg. The liver histology at autopsy showed active hepatitis probably due to coinfection with HCV.

Despite the high prevalence of HCV coinfection, the survival rate in hepatitis A, B, and NANB in this series was 1/2 (50%), 6/9 (67%), and 8/16 (50%), respectively. This very high survival rate in cases of NANB is remarkable and may have been due to the use of the ALSS. ALSS appears to sustain the patient free of complications sufficiently long until the liver is able to regenerate and regain adequate function to sustain life. An average duration of ALSS use of 19.3 days for survivors and 32.4 days for nonsurvivors stands in marked contrast to the 3, 4, and 8.6 days for ET, charcoal, and PAN HDF (6, 7, 8) reported by others in similar types of cases.

The high survival rate in the cases with NANB and acute exacerbation of HB carriers may be attributable to the use of IFN. It is generally believed that the HB virus is eliminated at the onset of FHF as a result of the host immune response (25, 26). In NANB hepatitis, however, replication of the responsible virus persists, as shown by the findings of HCV RNA in seven of the 14 NANB patients tested in this study.

Persistent viral replication in such cases presum-

ably allows continued liver cell necrosis to occur and renders FHF caused by HCV intractable to current clinical management. IFN may play a role in related HCV and may help to induce viral clearance.

The only treatment that may have helped the seven patients who died in this series that was not used is liver transplantation. Unfortunately this therapy is not available in Japan currently.

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