Clinical Application of Bioartificial Liver Support Systems

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Objective: To review the present status of bioartificial liver (BAL) devices and their obtained clinical results.

Background: Acute liver failure (ALF) is a disease with a high mortality. Standard therapy at present is liver transplantation. Liver transplantation is hampered by the increasing shortage of organ donors, resulting in high incidence of patients with ALF dying on the transplantation waiting list. Among a variety of liver assist therapies, BAL therapy is marked as the most promising solution to bridge ALF patients to liver transplantation or to liver regeneration, because several BAL systems showed significant survival improvement in animal ALF studies. Until today, clinical application of 11 different BAL systems has been reported.

Methods: A literature review was performed using MEDLINE and additional library searches. Only BAL systems that have been used in a clinical trial were included in this review.

Results: Eleven BAL systems found clinical application. Three systems were studied in a controlled trial, showing no significant survival benefits, in part due to the insufficient number of patients included. The other systems were studied in a phase I trial or during treatment of a single patient and all showed to be safe. Most BAL therapies resulted in improvement of clinical and biochemical parameters.

Conclusions: Bioartificial liver therapy for bridging patients with ALF to liver transplantation or liver regeneration is promising. Its clinical value awaits further improvement of BAL devices, replacement of hepatocytes of animal origin by human hepatocytes, and assessment in controlled clinical trials.

(*Ann Surg* 2004;240: 216–230)

Mortality of acute liver failure (ALF) remains high despite maximal supportive intensive care treatment. Mortality ranges from 60% to 90% depending on the cause of underlying liver disease. Survival of patients with ALF caused by acute hepatitis B is 12% to 23% in Western Europe.[1] Since the 1950s, several therapies to assist the failing liver have been introduced. These therapies range from drug treatment to liver support devices and liver transplantation. At present, standard treatment of ALF is orthotopic liver transplantation (OLT). Emergency OLT is associated with a 1-year survival of 60% to 90%, depending on the cause of ALF and the selection criteria applied for OLT.[1–7] However, due to the shortage of donor livers, a considerable number of patients with ALF die while on the waiting list for OLT. Despite the efforts to increase the donor liver pool by using split livers, living related donor livers, and marginal livers, the availability of donor livers is far less than the demand. In the United States at the end of 2001, 18,500 patients were waiting for OLT. In this year, 5250 out of 25,750 patients (20%) received a donor liver, whereas 1978 (7.7%) patients with hepatic failure died while waiting for OLT.[7] Of the high urgency patients (category I), 14% (97 out of 695) died while waiting for a donor liver. The median waiting time for a donor liver in this group was 10 days.[7]

Because of these high mortality rates and the increasing waiting times for transplantation over the last years,[7] there has been renewed interest in techniques for providing temporary liver support to bridge the patient with liver failure to OLT or liver regeneration. These techniques can be grossly divided into nonbiologic and biologic liver support.

NONBIOLOGIC LIVER SUPPORT

Lower and middle molecular weight toxic substances have been thought to play a crucial role in ALF. These water-soluble and protein-bound toxins cause multiple organ failure and hepatic encephalopathy, leading to coma and eventually to death. Many attempts have been made to develop nonbiologic liver support therapies based on detoxification of the patient's blood.[8–12] These therapies and their effects are summarized in Table 1. In the 1950s, hemodialysis was introduced in an attempt to remove toxins; however, no improvement of survival was achieved.[13–17] Hemofiltration, continuous convective solute removal across a permeable membrane, showed limited outcome.[18,19] Only case reports were published concerning hemodiafiltration, convection (large molecule), and diffusion (small molecule) removal across a membrane. These case reports showed improved bio-

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ISSN: 0003-4932/04/24002-0216

DOI: 10.1097/01.sla.0000132986.75257.19

TABLE 1. Nonbiological Liver Support

Nonbiological Liver Support	Reference	Technique	Outcome
Hemodialysis	13–17	Exchange diffusion across a semipermeable membrane between blood and a dialysis fluid	Improved coma, no improved survival
Hemofiltration	18,19	Continuous convective solute removal across a permeable membrane	Limited outcome
High volume plasmapehersis	45,46	Exchange of high plasma volumes	Improvement biochemical parameters and clinical status
Hemodiafiltration	18,20,21	Convection (large molecules) and diffusion (small molecules) removal across a membrane	Case reports, improved biochemical parameters and neurological status
Hemoperfusion	25	Perfusion of blood/plasma over charcoal, synthetic neutral resins, or anion exchange resins	Removal of toxins, improvement of mental status, no survival benefit
Hemodiabsorption	26	Dialysis against a combination of charcoal and cation exchanger	Improvement biochemical parameters and clinical status, no improved survival
Molecular Adsorbent Recirculating System (MARS)	29,31,32	Removal of protein-bound and water-soluble substances across a specialized albumin impregnated membrane against albumin rich recirculating dialysate	Improvement biochemical parameters and clinical status, significant survival benefit for subgroup of patients
Albumin dialysis system	33	Hemodiafiltration using albumin dialysate without recirculation	Improvement biochemical parameters and clinical status
Artificial Liver Support System (ALSS)	34	Combination of plasma exchange, charcoal hemoperfusion, plasma bilirubin absorption, charcoal plasma perfusion, hemofiltration and hemodialysis	Improvement biochemical parameters and clinical status
PF-Liver Dialysis	35	Combines hemodiabsorption with push-pull sorbent-based pheresis	Improvement biochemical parameters and clinical status

chemical parameters and neurologic status.[18,20,21] By hemo- and plasma perfusion, a more aggressive removal of toxic molecules that are protein bound was undertaken.[22] Various types of resins have been used,[23,24] especially effective in removal of lipophilic substances. Considerable experience has been obtained with activated charcoal as an adsorbent of possible toxins. However, the conclusion finally had to be drawn from controlled studies that these techniques did not improve survival.[25] Hemodiabsorbtion, dialysis against a combination of charcoal and cation exchanger, showed improved biochemical parameters and clinical status, but survival did not improve.[26] The nonspecific target of this technology was thought to be one of the reasons for its limited success.[27,28]

The most promising nonbiologic support therapies combine detoxification of water-soluble and protein-bound toxins in a dialysis system, such as the Molecular Adsorbents Recirculating System (MARS),[29–32] the albumin dialysis system,[33] the Artificial Liver Support System (ALSS),[34] and PF-Liver Dialysis.[35] Beneficial effects on plasma toxin levels were observed in noncontrolled studies of the albumin dialysis system, ALSS, and PF-Liver Dialysis systems in patients with liver failure. Only MARS treatment until now showed significantly improved survival in a controlled trial of a subgroup of patients with hepatorenal syndrome. Mortality rates in the control group were 100% at day 7 compared with 63% of the MARS-treated group.[31] In ALF patients, none of these systems have significantly improved survival.

In short, 1 or more nonbiologic liver support therapies may have shown benefit for short-term liver support in moderately affected patients with ALF; however, their unspecificity of removal of compounds and their lack of capacity to synthesize liver specific proteins and other hepatotrophic factors probably accounts for their limited effect. The success of OLT has demonstrated the importance not only of detoxification, but also metabolic functions in patient outcome. Because these functions can be carried out by hepatocytes, more is expected from biologic liver support systems.

BIOLOGIC LIVER SUPPORT

Biologic approaches rely on the functionality of livers or hepatocytes from xenogeneic or human origin that can be exploited to support the patient's liver (Table 2). These

TABLE 2. Biological Liver Support

Biological Liver Support	Reference	Technique	Outcome
Blood xeno cross-hemodialysis	37	Patient's blood dialyzed against blood of a living animal	Beneficial to patient, not suitable for further clinical application
Tissue xeno cross-hemodialysis	38,39	Patient's blood dialyzed against animal liver tissue preparations	Beneficial to patient, not suitable for further clinical application
Xenogeneic extracorporeal liver perfusion	9,42,43	Patient's blood perfused through an animal liver	Safe and provides metabolic support to the comatose ALF patient
Human cross-circulation	43	Shunt between patient's blood and blood of healthy human	Beneficial to patient, but harmful for donor
Exchange transfusion	44,45,47	Replace patient's plasma by healthy human plasma	Reversal of hepatic coma, large amount of normal plasma needed
Hepatocyte transplantation	48	Transplantation of isolated human hepatocytes in the patient's spleen or peritoneal cavity	Not much known in ALF patients, beneficial to patients with inborn metabolic errors, survival improvement in animal studies
BAL	See this review	Patient's blood or plasma perfused through an extracorporeal bioreactor filled with hepatocytes	Beneficial to patients, improvement of clinical and biochemical parameters, significant survival improvement in animal studies and subpopulation of human ALF

BAL, bioartificial liver; ALF, acute liver failure.

functions comprise detoxification, several metabolic functions, and synthesis of proteins and other molecules.

In 1956, it was demonstrated that fresh bovine liver homogenate could be used to metabolize salicylic and barbituric acids and keton bodies and produce urea from ammonium chloride.[36] The many different biologic approaches that followed thereafter comprised xeno cross-hemodialysis, in which the patient's blood was dialyzed against blood of a living animal[37] or animal liver tissue preparations.[38,39] Although these techniques could be beneficial to patients with liver failure, they were not considered to be suitable for clinical application because of the complexity of the procedure or rapid loss of effectivity. Moreover, xenogeneic extracorporeal liver perfusion in humans temporarily had been shown to improve biochemical parameters and the patient's clinical neurologic condition.[40,41] However, controlled clinical trials indicating survival improvement have as yet not been reported.[9,42] Liver support could be provided by human cross-circulation,[43] but the potential toxicity and adverse reactions in the donor severely limited this approach. Another approach, exchange transfusion was associated with reversal of hepatic coma.[44–46] In combination with hemodialysis, survival increased from 18% to 50% (4 out of 8 patients) in a noncontrolled study.[47] A major problem with exchange transfusion is the need of a large amount of normal plasma. Furthermore, this technique might at the same time remove essential factors, such as hepatotrophic factors.[47]

Isolated liver cells have been used in a variety of configurations: suspended, substrate attached, and encapsulated in semipermeable membranes. Hepatocytes used for liver support can be divided into 2 categories: implantable systems and extracorporeal systems. Several case reports and case series concerning transplantation of human hepatocytes show beneficial effects in liver failure.[48] Use of xenogeneic hepatocytes for hepatocyte transplantation in patients is not yet reported. Hepatocyte transplantation in the peritoneal cavity and spleen showed prolonged survival in animals with ALF,[49] but only if the transplantation occurred several days before induction of ALF.[50,51] Furthermore, ongoing hepatocyte injury by viral or toxic agents may not allow donor hepatocytes to organize into normal parenchymal architecture.[52]

Problems with blood clotting and immune reactions in extracorporeal whole liver perfusion[53] resulted in the development of BAL or hybrid liver support devices. The BAL systems are extracorporeal systems temporarily connected to the circulation of the patient. Bioartificial liver systems consist of an artificial component, i.e., the bioreactor and its equipment, and a biocomponent, i.e., hepatocytes. Although an increasing number of BAL devices have been produced or are currently under development, only 11 different BAL devices have, to date, been applied clinically. Significant prolongation of survival has been shown in animal studies with BAL systems,[54–58] and, therefore, clinical application of a BAL has high expectations. Herein, we review the 11 clinically applied BAL systems and the clinical results obtained with these devices.

CLINICALLY APPLIED BIOARTIFICIAL LIVER DEVICES

In 1987, Matsumura et al[59] reported the first application of a BAL support system in a patient. The principle of this BAL system was hemodialysis with a flow of 145 mL/min

against a suspension of 10 x 10^9 functioning, cryopreserved rabbit hepatocytes. The blood of the patient was separated from the rabbit hepatocytes by a cellulose membrane, which was permeable to low and middle molecular weight molecules. The bioreactor was placed between the radial artery and basilic vein. This case report described a 45-year-old male patient in hepatic failure due to an inoperable bile duct carcinoma that involved the bifurcation of the common hepatic duct. The patient underwent 2 treatments, lasting for 5 and 4.5 hours, respectively, and he survived with no signs of adverse events.

Two years later, Margulis et al[60] reported a controlled study including 126 patients in which a BAL device was used containing 40 x 10^6 porcine hepatocytes in a 20 mL polychlorovinyl capsule. The capsule contained a nylon filter in the outlet, which was filled with activated charcoal and granules of inorganic quartz glass. The capsule was incorporated into a forearm arteriovenous shunt. Each capsule was replaced by a fresh one every hour during a 6-hour treatment period. The blood flow through the bioreactor was 90 mL/min. Anticoagulation was obtained using heparin. Fifty-nine patients (20 hepatic coma and 39 prehepatic coma) were treated with this BAL device and were compared with a nontreated control group of 67 patients (30 hepatic coma and 37 prehepatic coma). In the control group, 27 patients (90%) died in the coma subgroup and 14 (38%) in the precoma subgroup. In the BAL-treated coma subgroup, 15 patients (75%) died, and the other patients (25%) initially regained consciousness, but died later due to progressive hepatic failure. In the BAL-treated precoma subgroup, 7 patients (18%) died, and the rest survived. Neurologic improvement was documented by clinical grading and electroencephalogram (EEG) monitoring. Overall, ammonia levels decreased with 50% compared with pretreatment levels. This BAL treatment was relatively simple and cheap.

No mention of Specified Pathogen Free (SPF) status of the animals used for hepatocyte isolation for the 2 abovementioned systems was made. No further reports concerning patient treatment with the Matsamura et al or Margulis et al systems have been published.

The systems that have been recently applied in the treatment of a number of patients are schematically presented in Tables 3 and 4.

The Extracorporeal Liver Assist Device

The Extracorporeal Liver Assist Device (ELAD)[29,61–65] (Houston, TX) is the only BAL device in which a human hepatocyte cell line (C3A) is used. The cell line is a clonal derivative of the hepatoblastoma cell line HepG2. The C3A cell line has been selected for use in the ELAD system because of its reduced tumorigenic potential and its high production of albumin and alpha-fetoprotein. The ELAD[64] consists of a dual pump dialysis system and hollow fiber cartridges containing C3A cells. A second cartridge can be connected in series if needed. Blood flows through the cartridge, and plasma is ultrafiltrated through the cellulose acetate fibers into the extracapillary space of the cartridge, where it comes in direct contact with the C3A cells. The semipermeable membrane, which separates the C3A cells from the blood, has a molecular weight cutoff of 70 kD. Therefore, no immunoglobulins or leukocytes come into direct contact with the C3A cells. Before the ultrafiltrate is returned to the bloodstream, it is passed through a dual membrane cell filter to prevent cells and cellular debris from entering the bloodstream. A disposable membrane oxygenator is used if the patient temporarily needs to be disconnected from the ELAD. A phase I trial[62] was performed in 11 patients: 10 with ALF and 1 with primary graft nonfunction (PNF). Cartridges maintained normal function during patient treatment of up to 58 hours, and their activity seemed to improve with blood perfusion. The only limitation to ELAD performance was clotting of the system. This has led to a more aggressive heparin treatment resulting in activated clotting times of 200 to 250 seconds. In this patient group, 4 patients were successfully bridged to OLT, 6 patients died before OLT, and 1 patient survived without OLT. Improvement in mental status occurred in 8 of the 11 patients. Most patients remained hemodynamically stable during ELAD treatment, and renal function was maintained in those patients who were not anuric at the start of treatment. No significant changes in vital signs, white blood cell count, or complement were noted. Several adverse events took place that were not related to ELAD treatment. However, 1 patient was noted with a short period of hypotension, which was corrected by fluid administration.

In a pilot controlled trial,[61] 24 patients were stratified into 2 study groups according to their predicted outcome. Group I (n = 17) comprised patients with ALF who were considered to have a substantial chance (30–50%) of spontaneous recovery. Patients in group II (n = 7) fulfilled criteria for liver transplantation at enrollment. The patients were randomized into 2 arms: in arm I, patients received standard therapy alone (control arm); in arm II, patients received ELAD support in addition to standard therapy. Six patients in group I, 3 in each arm, deteriorated and were put on the waiting list for liver transplantation. Survival in group I was 78% in the control arm and 75% in the ELAD arm. In group II, the survival rates were 25% and 33%, respectively. Eventually, 13 patients were put on the waiting list for liver transplantation. Six patients (46%) of this subgroup received a liver transplant, and 7 patients died without transplantation. Eleven patients, all in group I, survived without liver transplant. Assessment of serial changes in encephalopathy appeared to show some benefit with ELAD support. There was no significant difference in renal function between groups. Analysis of biochemical variables after ELAD treatment

TABLE 3. Characteristics of Eight Different BAL Systems

	ELAD	HepatAssist	TECA-HALSS	BLSS	RFB	LSS and MELS	AMC-BAL	HBAL
Center	Amphioxus Cell Technologies, Houston, TX	Cedars-Sinai Medical Center, Los Angeles, CA, and Circe Biomedical, Lexington, KY	Chinese PLA General Hospital, Beijing, China	Excorp Medical, Inc, Oakdale & University of Pittsburgh, PA	University of Ferrara and RanD, Cavezzo Italy	Charité, Humboldt University, Berlin, Germany	Academic Medical Center, University of Amsterdam, The Netherlands	Nanjing University, Nanjing, China
Cell type	Human, tumor derived	Porcine	Porcine	Porcine	Porcine	LSS → porcine MELS → human	Porcine	Porcine
Cell source	Cultured C3A	Cryopreserved	Freshly isolated	Freshly isolated	Freshly isolated	Freshly isolated	Freshly isolated	Freshly isolated
Cell amount	200–400 gram	$5–7 \times 10^9$	$10–20 \times 10^9$	70–120 g	200–230 g	Up to 600 g	10×10^9	10×10^9
Device function pretreatment	Not reported	Not reported	Not reported	Not reported	Not reported	LSS → not reported MELS → yes	Urea synthesis	Not reported
Device sterility pretreatment	Not reported	Not reported	Not noted	Microbial culture	Not noted	LSS → not reported MELS → yes	PCR and microbial culture	Not reported
Mass transfer	70 kD cutoff membrane	0.2 μm porous membrane	Membrane, details not reported	100 kD cutoff membrane	Polyester screen, cutoff 1 μm	LSS → 300 kD cutoff MELS → 400 kD cutoff	Direct hepatocyte–plasma contact	100 kD cutoff membrane
Barrier filter	1 μm	No	Not reported	No	0.4 μm	No	Leukocyte filter and 0.47 μm filter	No
Plasma/blood perfusion	Blood	Plasma	Plasma	Blood	Plasma	Plasma	Plasma	Plasma
Blood filtration rate	150–200 mL/min	90–100 mL/min	Not reported	100–250 mL/min	80 mL/min	150–300 mL/min	100 mL/min	100 mL/min
Plasma filtration rate	Not applicable	50 mL/min	Not reported	Not applicable	22 mL/min	31 mL/min	40–50 mL/min	Not reported
Bioreactor flow rate	15–200 mL/min	400 mL/min	Not reported	100–250 mL/min	1.0–1.5 mL/min/g hepatocytes	100–200 mL/min	150 mL/min	Not reported
Treatment time	Up to 168 hrs	6 hrs	Up to 5 hrs	12 hrs	Maximal 24 hrs	LSS → 7–46 hrs MELS → 7–74 hrs	Maximal 24 hrs	6 hrs
Oxygenation level	Prebioreactor	Prebioreactor	Not reported (prebioreactor)	Prebioreactor	Prebioreactor	Local, inside bioreactor	Local, inside bioreactor	Not reported
Oxygenation gas	Not reported	Not reported	Not reported	Mixture $O_2/CO_2/N_2$, → pH and O_2 guided	95% O_2/5% CO_2	Not reported	95% O_2/5% CO_2	Not reported
Anticoagulation	Heparin	Citrate	Heparin	Heparin	Heparin/citrate	Heparin	Heparin	Not reported
Extra detox devices	No	Charcoal column prebioreactor	Charcoal column prebioreactor	No	No	LSS → no MELS → albumin dialyzation	No	Hetrogeneous, (charcoal or bilirubin column)

BAL, bioartificial liver; ELAD, Extracorporeal Liver Assist Device; TECA-HALSs, TECA-Hybrid Artificial Liver Support System; BLSS, Bioartificial Liver Support System; RFB, Radial Flow Bioreactor; LSS, Liver Support System; MELS, Modular Extracorporeal Liver Support; AMC-BAL; AMC-Bioartificial Liver; HBAL, Hybrid Bioartificial Liver; PCR, polymerase chain reaction.

TABLE 4. Clinical Outcome of Eight Different BAL Systems

	ELAD	HepatAssist	TECA-HALSS	BLSS	RFB	LSS and MELS	AMC-BAL	HBAL
Type of trial	1. Safety evaluation 2. Controlled trial	1. Safety evaluation 2. Controlled trial	Safety evaluation	Safety evaluation	Safety evaluation	Safety evaluation	Safety evaluation	Safety evaluation
No. of patients	1. 11 2. 24	1. 10 2. 171	6	4	7	1. LSS (porcine) → 7 2. MELS (human) → 8	12	12
Indication of BAL treatment	1. ALF 10 PNF 1 AOC 0	1. ALF 7 PNF 1 AOC 2	ALF 3 PNF 0 AOC 3	ALF 2 PNF 0 AOC 2	ALF 4 PNF 3 AOC 0	1. LSS → not reported 2. MELS → ALF 2 PNF 2 AOC 4	ALF 12 PNF 0 AOC 0	ALF 12 PNF 0 AOC 0
1. safety study, 2. controlled trial	2. ALF 24* PNF 0 AOC 0	2. ALF 147 PNF 24 AOC 0						
Bridge to OLT and survival of OLT patients	1. Bridge to OLT 4 Survival no OLT 1 Died no OLT 6	1. Bridge to OLT 8 Survival no OLT 0 Died no OLT 2	Not reported, at least 2 patients survived without OLT	No OLT waiting list: 3 deaths 5–10 days post-BAL, 1 survival → auxillary transplant	Bridged to OLT 6 Survival no OLT 0 Died before OLT 1	1. Bridged to OLT 6 Survival no OLT 0 Died before OLT 0	Bridge to OLT 11 Survival no OLT 1	Bridged to OLT 0 Survival no OLT 9 Died post BALT 3
1. safety study, 2. controlled trial	2. Bridge to OLT 6† Survival no OLT 0 Died no OLT 7	2. Bridge to OLT n.r Survival no OLT n.r Died no OLT n.r				2. Bridged to OLT 6 Survival no OLT 1 Died before OLT 0 Died no OLT 1		
Complications during treatment	Hypotension in 1 patient; 2 patients → treatment discontinued due to bleeding and pyrexia	Occasionally hypotension	No	Transient hypotension	No, treatment in 2 patients was discontinued due to external reasons	LSS → not reported MELS → no	2 × short period of hypotension, easily corrected → treatment continued	No
Survival improvement	No	Only in subgroups improved survival was found (n = 171)	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Neurological improvement	Probably	Yes	Yes	No, possible due to sedation	Yes	LSS → not reported MELS → yes	Yes	Unclear
Ammonia elimination	−8% (increased)	18%‡	Not reported	33%	33%	1. No improvement 2. Not reported	44%	Unclear
Total bilirubin elimination	−20% (increased)	18%‡	Not reported	6%	11%	1 & 2 Not reported	31%	Unclear
Tested for PERV	Not applicable	Yes → negative	Not reported	Yes → negative	Yes → negative	LSS → Yes, negative MELS not applicable	Yes → negative	Not reported

Average ammonia and total bilirubin elimination percentage is represented as a decrease of concentration after bioartificial liver transplantation treatment compared to the concentration before treatment.

*Only orthotopic liver transplantation waiting list patients included.

†Two groups of patients (see text, ELAD).

‡Average of data published in 5 different papers.[67,70,71,73,74]

BAL, bioartificial liver; ELAD, Extracorporeal Liver Assist Device; TECA-HALSS, TECA-Hybrid Artificial Liver Support System; BLSS, Bioartificial Liver Support System; RFB, Radial Flow Bioreactor; LSS, Liver Support System; MELS, Modular Extracorporeal Liver Support; AMC-BAL, AMC-Bioartificial Liver; HBAL, Hybrid-Bioartificial Liver; ALF, acute liver failure; PNF, primary graft nonfunction; AOC, acute-on-chronic liver failure; OLT, orthotopic liver transplantation; BALT, bioartificial liver transplantation.

showed an increase of plasma ammonia (8%) and bilirubin (20%) concentrations compared with corresponding values before ELAD treatment; other variables measured were not influenced by ELAD treatment.

Few adverse events occurred in the controlled trial. Two patients were withdrawn from the study. One patient became tachypneic, tachycardic, and pyrexial. These phenomena resolved rapidly after discontinuing the hemoperfusion. The second patient developed overt bleeding because of an exacerbation of pre-existing disseminated intravascular coagulation. After stopping the perfusion and infusion of fresh-frozen plasma, bleeding ceased and the platelet count increased. No severe hypotension was observed in this controlled trial. In all, this controlled trial did not demonstrate a significant difference in survival between ELAD treated patients and controls. Furthermore, no improvement of biochemical parameters was observed.

Recently, a new clinical trial with a slightly modified version of the ELAD system has started and was preceded by a phase I trial in 5 patients[66] (not listed in Tables 3 and 4). The new system uses ultrafiltrated blood instead of whole blood generated by a 120 kD cutoff membrane (instead of 70 kD). Four cartridges with approximately 100 g of C3A cells were used for each treatment. The flow rate through one cartridge is 500 mL/min instead of 150 to 200 mL/min. Oxygen and glucose consumption are frequently monitored to ensure metabolic activity of the cells in the cartridges. The 5 patients, all candidates for OLT, entered into an open-label, randomized, controlled pilot multicenter study of approximately 24 patients with clinical diagnosis of ALF or primary PNF. The treatment period ranged from 12 to 107 hours. No adverse events were observed during modified ELAD treatment. Ammonia and lactate plasma concentrations were not influenced by the ELAD treatment. Bilirubin plasma levels were not mentioned. Four patients were successfully bridged to OLT. One patient died within 2 days after OLT due to infection and deterioration of neurologic status.

The HepatAssist System

The HepatAssist BAL device[67–77] developed in Los Angeles, CA, by Demetriou et al has been tested in the largest controlled clinical trial of a BAL device. The biologic component consists of 5 to 7 x 10^9 cryopreserved porcine hepatocytes. The microcarrier-attached cells are inoculated into the extrafiber space of a hollow fiber bioreactor. After plasma separation, plasma of the patient first passes over an activated charcoal-coated cellulose column and through an oxygenator before it is circulated through the semi-permeable fibers (Ø 0.2 μm) in the hollow fiber bioreactor. After passing the bioreactor, treated plasma and the blood cells are reconstituted and returned to the patient.

In a phase I safety evaluation study,[67,76] 9 adult patients and a 10-year-old boy were treated with the HepatAssist system. Nine patients had ALF, and 1 had PNF. Eight patients were successfully bridged to OLT and 2 patients died without OLT. The pediatric patient was successfully bridged to OLT. In 6 patients who had deep coma with brain edema and intracranial hypertension, a rapid normalization of intracranial pressure (ICP) was observed during treatment. Blood ammonia levels decreased in all patients by 36% on average. The mean total bilirubin concentration decreased by 11%. Treatments were well tolerated. No reactions to porcine hepatocytes were observed, and all patients remained hemodynamically stable throughout the treatment period.

In an uncontrolled follow-up study, 39 ALF patients classified in 3 groups were treated with the HepatAssist BAL.[70] Group I (n = 26) patients fulfilled the criteria of ALF and were candidates for OLT. Group II (n = 3) patients had undergone OLT and had PNF. Patients in group III (n = 10) presented with acute on chronic liver disease and were not candidates for OLT. In group I, 18 patients (69%) were successfully bridged to OLT, of which 17 patients completely recovered. One patient died 7 days post-OLT due to PNF. Six patients (23%), of whom 5 had acetaminophen-induced ALF, recovered spontaneously after BAL treatment without OLT. Two patients (8%) were removed from the transplant waiting list because of initial clinical improvement during BAL treatments, but they finally died 21 and 44 days after the start of BAL treatment. All 3 patients in group II were successfully bridged to OLT and fully recovered. All patients in group III exhibited transient clinical improvement after BAL treatment; however, 8 patients (80%) died 1 to 21 days after first BAL treatment.

HepatAssist treatment was associated with improvement in neurologic status,[69,70] ICP, and Glasgow Coma Scale (GCS). Biochemical parameters improved in all 3 groups. The mean total bilirubin concentration in group I decreased by 18% of the concentration at the start of treatment. Mean ammonia levels also decreased by 18% of the initial level. Data on above-mentioned biochemical parameters were obtained from 5 different publications[67,70,71,73,74] for the purpose of this review. Presumably, several patients have been taken into account more than once in these publications.

In 1 patient, transient hypotension was observed after which treatment was discontinued. No other adverse events were noted. The HepatAssist system was shown to be safe, well tolerated by the patients, except for hypotension in 1 patient, and provided temporary physiologic support to patients with ALF. Patients were tested retrospectively for porcine endogenous retrovirus (PERV). There was no evidence of viral transmission from pig cells to the patients. The positive outcomes in this uncontrolled study provided the incentive to conduct a randomized, controlled clinical trial.

In this trial, 171 patients (147 ALF and 24 PNF)[77] were randomized into a BAL treatment arm (n = 85) and a control arm (n = 86). The primary end point was 30-day survival;

this was achieved in 71% in the BAL treatment arm and 62% in the control arm ($P = 0.28$). In the ALF subgroup, the 30-day survival was 73% and 59% for the BAL and control group, respectively ($P = 0.10$). However, a significant survival advantage of 33% (37% in the control group versus 70% in the BAL group; $P = 0.018$) was associated with BAL treatment of acetaminophen overdose (n = 39). Extension of this clinical trial is planned.

TECA-Hybrid Artificial Liver Support System

The TECA-Hybrid Artificial Liver Support System (TECA-HALSS)[78,79] developed in Beijing, China, consists of an extracorporeal hollow fiber bioreactor loaded with 10 to 20 x 10^9 porcine hepatocytes. The hepatocytes circulate in suspension through the outer space of the hollow fibers in the bioreactor. Plasma is perfused through the fibers of the bioreactor. After perfusion through a charcoal filter and the bioreactor, the plasma is reconstituted with the blood cells and then returned to the patient. The treatment lasts for a maximum of 5 hours per bioreactor.

Six patients, 3 with ALF and 3 with acute-on-chronic liver failure, were treated with the TECA-HALSS system.[78] During treatment, vital signs remained stable, and no thrombosis or bleeding events were noted. Neurologic improvement occurred in those patients entering with drowsiness or coma. After TECA-HALSS treatment, ammonia concentrations were substantially lower. In 1 patient, the ammonia level decreased by 31% and total bilirubin concentration decreased by 15%. Unfortunately, no mean data for the whole group are available. Neither was additional information on safety reported.

The Bioartificial Liver Support System

The Bioartificial Liver Support System (BLSS) device,[80,81] developed in Pittsburgh, PA, uses 70 to 120 g of primary porcine hepatocytes. The hepatocytes, mixed with 20% collagen, are housed in an extrafiber space. Whole blood is perfused through the fibers of the bioreactor after passing through an oxygenator. Mass transfer depends on diffusion across a semipermeable fiber membrane with a 100 kD cutoff.

Four patients were treated with the BLSS in a phase I clinical trial.[81] Causes of ALF in this group were acetaminophen intoxication, Wilson disease, acute alcoholic hepatitis, and chemotherapy. Survival outcome was not mentioned. Mean ammonia levels decreased by 33% compared with pretreatment levels and total bilirubin concentration decreased by 6%. Renal function and neurologic function did not seem to be influenced by BLSS perfusion. In 1 patient, transient hypotension at the start of the BLSS perfusion was observed. This adverse event was easily corrected by fluid administration. No PERV transmission was detected by examination of lymphocytes up to 12 months after BLSS treatment.

The Radial Flow Bioreactor

The Radial Flow Bioreactor (RFB),[82,83] developed in Ferrara, Italy, comprises a woven-nonwoven polyester matrix sandwiched between 2 precision woven polyester screens. About 200 g of primary porcine hepatocytes are injected into the 6-mm-thick polyester matrix. The 2 polyester screens prevent hepatocytes leaking out of the bioreactor during perfusion of the plasma of the patient. Oxygenation of the cells is accomplished by perfusion of the plasma through an oxygenator before it enters the bioreactor. During RFB treatment, the function of the bioreactor is evaluated by determining its oxygen consumption. Oxygen consumption by the hepatocytes in the bioreactor decreases during treatment of a patient, indicating exhaustion of the hepatocytes.

Seven patients waiting for OLT were included in a phase I safety evaluation trial.[83] The causes of ALF were viral hepatitis in 3, PNF in 3, and abdominal trauma in 1 patient. Six out of the 7 patients underwent OLT within 2 to 6 hours after completion of the RFB treatment. Five out of 6 patients survived after OLT. One patient with PNF eventually was not a candidate for retransplantation and died of multiorgan failure (MOF). Late death occurred in the trauma patient due to MOF after retransplantation. Treatment was associated with amelioration of neurologic dysfunction. Radial Flow Bioreactor treatment lowered the mean ammonia and bilirubin level by 33% and 11%, respectively. Radial Flow Bioreactor treatments were well tolerated, and patients remained hemodynamically stable throughout treatment. No adverse events were observed during or after the treatment. Porcine endogenous retrovirus transmission from the porcine cells to mononuclear cells was not detected during short-term follow-up.

The Hybrid Liver Support System and Modular Extracorporeal Liver Support

The Liver Support System (LSS) device,[84–86] developed in Berlin, Germany, consists of an especially designed bioreactor aiming at improving cell oxygenation and mass exchange. The system consists of interwoven hollow fiber membranes, creating a 3-dimensional framework over which hepatocyte aggregates are distributed. Three bundles of hollow fibers are situated inside the bioreactor. Two of these bundles consist of hydrophilic fibers (300 kD cutoff) and are used for plasma perfusion. By closing 1 end of each bundle, plasma entering the bioreactor enters the extracapillary space via one fiber bundle, makes contact with the hepatocytes, and leaves the bioreactor via the second fiber bundle. The third bundle of hollow fibers is made of hydrophobic membranes and is used for gas exchange inside the bioreactor. The bioreactor contains up to 500 to 600 g of hepatocytes. The

LSS is the only system that has been used in clinical studies with primary porcine hepatocytes as well as primary human hepatocytes derived from discarded donor livers.[87]

Seven ALF patients, with coma stage II to IV, were treated for 8 to 46 hours with the porcine cell-based LSS system.[86] All patients were successfully bridged to OLT. Elevated plasma ammonia levels were not corrected by LSS treatment. No data concerning clinical parameters, total bilirubin, and adverse events have been reported. Porcine endogenous retrovirus transmission was tested negative.[88]

In a phase I study with primary human hepatocytes, 8 patients were treated with the Modular Extracorporeal Liver Support (MELS) system. The MELS concept combines different extracorporeal therapy units, tailored to suit the individual clinical needs of each patient.[87] The MELS consists of the LSS system combined with a DetoxModule based on single-pass albumin dialysis for removing albumin-bound toxins. Human hepatocytes were harvested from donor livers that were discarded because of steatosis, cirrhosis, fibrosis, or mechanical injury. Two patients with ALF (not further specified), 2 patients with acute-on-chronic liver failure, and 2 patients with PNF were successfully bridged to OLT. The other 2 patients suffered from acute-on-chronic liver failure and were not candidates for OLT due to continuing alcohol consumption. One of these 2 patients died 3 weeks after MELS treatment. The overall MELS treatment time in this group ranged from 7 to 144 hours. The longer treatments were performed with 2 consecutive bioreactors. No adverse events were observed. In all 8 cases, neurologic status improved, and slight improvement of coagulation was observed during treatment.

The AMC-Bioartificial Liver

The AMC-Bioartificial Liver (AMC-BAL),[57,58,89–96] developed in Amsterdam, the Netherlands, consists of a hollow fiber bioreactor (polysulfon housing) and a plasmapheresis system. At least 10×10^9 viable porcine hepatocytes are attached in a 3-dimensional configuration to a nonwoven hydrophilic polyester matrix. The matrix, with a thickness of 4 mm and a total surface area of 5610 cm^2, is spirally wound around a massive core. In between the layers of the matrix, hollow fibers for on-site gas exchange are positioned in a longitudinal direction. The blood of the patient undergoes a plasma filtration treatment, after which the resulting plasma is perfused through the bioreactor and again reunited with the blood cells. The most noteworthy features of the AMC-BAL are the direct contact between small aggregates of hepatocytes and the plasma of the patient, resulting in optimal mass transfer, and direct oxygenation of the hepatocytes.

Seven patients were treated with the AMC-BAL in a phase I safety evaluation trial in Naples and Rome, Italy.[95] All patients had hyperacute (n = 6) or acute (n = 1) liver failure according to Crepaldi et al[97] and met the criteria for OLT.[98,99] All patients had grade III to IV encephalopathy. The cause of ALF in 3 patients was acute hepatitis B, acute hepatitis A in 1 patient, and acute fatty liver of pregnancy in 1 patient. In 2 patients, the cause of liver failure was not determined. Duration of total AMC-BAL treatment ranged from 8 to 35 hours. Three patients received serial treatment with 2 BALs. Six of the 7 patients were successfully bridged to OLT. One patient recovered after 2 BAL treatments over an interval of 3 days without OLT. Two patients died of complications post-OLT. One patient died 1 day after OLT due to PNF of a marginal donor liver. The second patient died 14 days after OLT due to mesenteric thrombosis that resulted in massive bowel infarction. Treatment of all patients was associated with an improved neurologic state and stabilization of hemodynamics. Improved urine output was noted in patients with renal insufficiency. After AMC-BAL treatment, the average plasma ammonia and bilirubin levels decreased by 44% and 31%, respectively.[95] In 2 patients, a short period of hypotension was observed after connection to the BAL system. This hypotension was easily corrected by dopamine and fluid administration. No other adverse events were observed in these patients. None of the treated patients were positive for PERV.[95]

Five additional patients with ALF were included in the phase I trial and treated with the AMC-BAL (unpublished data). All 5 patients were successfully bridged to OLT. One patient died 1 day after OLT due to postoperative bleeding and PNF. A second patient, with a GCS of 3, died 2 weeks after OLT due to multiorgan failure. In summary, 12 patients have been treated with the AMC-BAL in a phase I trial. Eleven patients were successfully bridged to OLT, and 1 patient survived after 2 treatments without OLT. Four patients died within a month after OLT due to disease and OLT-related problems. The 8 other patients (66%) are in good health at the moment and have post-BAL survival times ranging from 6 to 30 months.

The Bioartificial Hepatic Support System

Concerning the Bioartificial Hepatic Support (BHS)[100] system, developed in Udine, Italy, only 1 case has been published. This system is not represented in the accompanying tables because of limited information about the system and the absence of a published phase I study. Fifteen billion cryopreserved porcine hepatocytes, together with 10 g hydrated collagen-coated dextran microcarriers, were loaded in the extracapillary space of a hollow fiber bioreactor with a porosity of 0.6 μm. After plasmapheresis, the plasma of the patient passed a cellulose-charcoal column, an oxygenator, and a heater before entering the bioreactor with a flow not further defined.

A 56-year-old patient with acute-on-chronic liver failure (HBV cirrhosis) and grade III portosystemic encephalopathy was treated with the BHS system.[100] The patient under-

went 3 treatments lasting 6 hours each, with 48-hour intervals. Pulmonary and cardiovascular functions remained stable, and the patient tolerated the procedures well. Bilirubin as well as ammonia concentrations improved during treatment. Temporary neurologic improvements were observed after each BHS treatment. The patient was not a candidate for OLT and died 13 days after the last BHS treatment.

Hybrid-Bioartificial Liver

Most recently, a Chinese group from Nanjing published a patient study with a BAL system called the Hybrid-Bioartificial Liver (HBAL).[101] No earlier in vitro, animal, or patient studies with the HBAL were published. The BAL consists of a polysulfon hollow fiber bioreactor with an internal volume of about 360 mL. More than 10 x 10^9 porcine hepatocytes cultured overnight in cell suspension were loaded into the extrafiber compartment of the bioreactor. Plasma was perfused through the hollow fibers with a membrane cutoff of 100 kD (same material and provider as the TECA-HALLS system).

In a phase I trial, 12 patients were treated with the HBAL system.[101] In some patients, the plasma was perfused through a charcoal column or through a bilirubin absorption column before entering the HBAL system, other patients received HBAL only, and in another 2 patients, plasma exchange was first performed 24 hours prior to HBAL treatment. In summary, a heterogeneous treatment regimen was described in this paper. All patients suffered from hepatitis B ALF. The ALF status or coma grade was not described in this patient group. No inclusion or exclusion criteria were mentioned. Hybrid-Bioartificial Liver treatment lasted 6 hours. Two patients received 2 consecutive treatments. No adverse effects were observed in the 12 patients; however, 3 of them died soon after the HBAL treatment. Nine patients were described as improved, but no clear data were presented in this paper.

DISCUSSION

Comparison of the clinically applied BAL devices is impaired by the variability in devices and cells, setup of the treatments, patients, and in the outcome parameters used. Nonetheless, some conclusions can be extracted from the variety of data derived from the different BAL systems, excluding the first 2 systems described by Matsumura et al[59] and Margulis et al[60] and the BHS system,[100] of which no recent data or very limited data were reported.

Bioartificial Liver Devices

The devices described here are, except for the RFB, hollow fiber devices, which differ in the mode of oxygenation, bidirectional mass exchange, and cell type or cell treatment. These are considered crucial parameters in the design of bioreactors, but other factors, such as an extracellular matrix environment and media composition, may also have considerable impact on level and stability of hepatocyte function.

It is often overlooked that BAL systems lack a biliary system for the excretion of conjugated bilirubin. Excretion of metabolites through bile depends on the remnant damaged liver mass. Hepatocytes in the bioreactor will produce bile acids and salts, most of them being toxic protein bound substances, and further increase the concentrations in blood.[102,103] Incorporation of a detoxification module into the BAL system (as in HepatAssist, TECA-HALSS, MELS, and HBAL) may lower the toxic burden for the hepatocytes in the bioreactor and for the patient. For bridging ALF patients to OLT, the combination of a BAL and an artificial detoxification module may therefore provide optimal conditions for the treatment of ALF patients, although removal of hepatotrophic factors should be prevented. The latter is especially important if the goal of BAL treatment is bridging of the patient to liver regeneration.

Bioactive Mass and Source of Hepatocytes

Bioactive mass and cell type or cell source play a key role in BAL treatment. The efficacy of the treatment depends on the bioactivity of the cells in the bioreactor. These cells should be able to take over function of the diseased or absent liver. One of the unsolved questions in BAL research is with which functions and at what level a BAL should compensate the diseased liver. It is known from partial hepatectomy studies that about 20% of healthy liver mass, which contains approximately 200 g or 20 x 10^9 hepatocytes, is needed to survive.[104–106] Thus, if no active liver mass is left in the patient, approximately 20 x 10^9 well-functioning hepatocytes are theoretically needed to keep the patient alive.

A variety of cell masses are used in the different BAL systems. Some groups express cell mass in grams, whereas others use cell number by cell count, which is confusing, particularly when it is not described how these figures are obtained. Therefore, it is difficult, if not impossible, to compare the bioactive mass between systems. Cell mass used in the current, clinically applied systems ranges from 5 x 10^9 cells (HepatAssist) to 600 g (LSS & MELS). Most systems use about 10 x 10^9 to 20 x 10^9 hepatocytes, which is, in theory, sufficient to support a liver with limited residual function. However, the viability and function of the cells prior to loading and after culture in the bioreactor may vary considerably and influence the effective biomass. These issues are not reported for the BAL systems, except for the AMC-BAL and MELS systems in which the urea producing capacity of the cells in the bioreactor, and in case of the MELS also other liver functions, was tested preceding connection to the patient.

Different cell types are used in the BAL systems currently under clinical study, each with specific advantages

and disadvantages[107] (Table 5). Because of their optimal function, primary human hepatocytes are the first choice for patient treatment, but their availability is low. The main source of primary human hepatocytes for BAL use is discarded donor livers. The primary human hepatocytes derived from discarded donor livers are characterized by heterogeneity and low viability.[87,108] Moreover, the logistics around discarded donor livers are complex. However, according to Gerlach et al, the number of discarded donor livers, approximately 20% to 25% of all explanted livers, corresponds to the number of patients with ALF who require bridging therapy to transplantation.[88] Alternatively, primary hepatocytes from animals, most often pigs, have been used in the majority of clinically applied bioreactors. Porcine livers are available in large quantities and can be obtained on demand. However, animal hepatocytes produce xenogeneic proteins, which may cause serum sickness within a week of repetitive treatments.[76,109,110] Such immunologic problems will not be expected if treatments with porcine-based artificial liver systems do not exceed 5 to 6 days. Additionally, the risk of zoonosis severely limits the clinical application of porcine-based BAL systems, because in many European countries, xenotransplantation-related treatments are prohibited. In 9 of the 11 systems reviewed, hepatocytes from xenogeneic origin were used in the treatment of about 150 patients. No clinically important zoonosis or virus transmission has been reported in these patients. An important measure to maximally reduce the risk of zoonosis is the use of SPF animals, which are bred under strict conditions and are checked for a wide range of pathogens on a frequent base. In 3 of the porcine cell-based systems (ie, TECA-HALLS, BHS, and HBAL), the SPF status of the source animals for hepatocyte isolation was not reported, which raises concerns regarding safety of these cells. On the other hand, endogenous retroviruses, like PERV, are incorporated in the pig genome and therefore are also present in SPF pigs. Therefore, the risk of zoonosis, although very limited, cannot be neglected in view of the supposed impact on public health. In 5 of the presented studies, PERV tests were included and proved negative. We would like to encourage all groups working with xenogeneic-based BAL systems to test patients as long as possible for PERV and other possible zoonosis.

Optimal preservation of primary hepatocytes is necessary to improve BAL availability and logistics. Most efforts have been put into cryopreservation of hepatocytes. Cryopreservation may, however, lead to decreased viability, cell attachment capacity, and function. These effects are most significant if porcine or human hepatocytes are cryopreserved in cell suspension[111,112] (unpublished data). Hepatocytes that are cultured[113–115] or attached to microcarriers[68,116,117] prior to cryopreservation maintain better cell function compared to preserved cell suspensions. From the BALs reported here, the HepatAssist system makes use of cryopreserved hepatocytes that are attached to microcarriers. In contrast, the BHS system uses cryopreserved hepatocyte suspensions. After thawing, the hepatocytes were combined with hydrated collagen-coated dextran microcarriers.

Most of the presented BAL systems rely on freshly isolated hepatocytes and therefore require optimal preservation conditions during transport of the loaded bioreactor from the laboratory to a usually remote center. One possibility is to preserve the hepatocyte-loaded bioreactor at 4°C. A standard organ preservation solution is generally used, such as University of Wisconsin solution or Celsior™. Before connection to the patient, the preservation solution is washed out of the bioreactor. It should, however, be taken into account that cold preservation causes loss of hepatocyte function inside the bioreactor.[118] Alternatively, the hepatocyte-loaded bioreactor can be perfused and oxygenated under (sub)normothermic conditions during transport. Under normothermic (37°C) conditions, cell function of the hepatocytes will stabilize as much as possible and will be comparable to that of a laboratory culture of the cells in the bioreactor. A normothermic transport system for BALs is therefore an attractive option and deserves further investigation.

Cell lines, most often derived from tumors, are also applied to BAL systems as an alternative for primary cells. However, the functionality and safety of these cells are a matter of discussion. The ELAD system is based on the C3A cells, which display a number of liver functions, such as

TABLE 5. Characteristics of Different Cell Sources in Relation to BAL Application

Cell Source	System	Availability	Logistics	Immunology	Tumorigenicity	Zoonosis	Function
Human primary	4	+/−	−	++	+	+++	++
Human cell line	1	+++	+++	+++	+/−	+++	+/−
Porcine primary	3, 4, 5, 6, 7, 9	++	+	+/−	+++	+/−	+++
Porcine (cryo)preserved	2, 8	+++	+	+/−	+++	+/−	+

1, Extracorporeal Liver Assist Device; 2, HepatAssist; 3, TECA-Hybrid Artificial Liver Support System; 4, Bioartificial Liver Support System; 5, Radial Flow Bioreactor; 7, AMC-Bioartificial Liver; 8, Bioartificial Hepatic Support; 9, Hybrid-Bioartificial Liver; −, poor; +++, very good.

albumin production, but, for example, their ammonia reducing capacity is very low. Detachment of the C3A hepatoma cells and their subsequent escape from the ELAD system to the blood stream of the patient is considered to be only a theoretical concern.[64] No tumorigenic infiltration of patients by C3A cells has been described so far.

Current advances in immortalization of hepatocytes and stem cell biology may offer better prospects, but have not yet been tested in a clinical setting.[119]

Patient Survival

Three of the described BAL systems (ie, Margulis BAL, ELAD, HepatAssist) have been tested in a controlled clinical trial. In these studies, there was no significant effect on survival. Only a subgroup of patients with ALF due to acetaminophen overdose, showed significant improvement in survival after treatment with the HepatAssist device.[77] Because BAL treatment in ALF patients is usually followed by urgent OLT, having a major influence on 30-day survival, a large number of patients is needed in a controlled trial to show efficacy and improved survival of a BAL treatment. Of the BAL systems described here, treatment with the HepatAssist,[54] LSS,[55] and AMC-BAL[57,58,92,93] significantly improved survival in animal models. The BLSS system showed a trend to improved survival in a galactosamine-induced ALF dog model.[80] The TECA-HALLS system shows beneficial outcome on survival in ALF dogs but *P* value for survival benefit was not reported.[79] The ELAD system has been tested in a small number of anhepatic dogs. Of 3 dogs, 1 lived longer (but not statistically significant) after ELAD treatment compared to a control group ($n = 3$).[120] No reports concerning safety and testing of efficacy in ALF animal models prior to clinical application are available of the other BAL systems (RFB, BHS, HBAL).

Apart from survival, the efficacy of the different BAL systems can also be assessed from the effects on neurologic status and blood chemistry.

Neurologic Improvement

Application of most systems was associated with neurologic improvement during and after treatment. In the LSS study, there was no report on the neurologic status of patients. However in the MELS system (primary human hepatocytes), it was reported that all patients improved in regard with neurologic status. In the BLSS study, it was not possible to measure neurologic changes due to sedation of the patient. In the other studies, neurologic improvements assessed by GCS and, in some studies, also by EEG and/or ICP measurement were associated with application of a BAL system.

Biochemical Improvement Following Bioartificial Liver Treatment

Biochemical improvement as a result of BAL treatment, as judged by elimination of ammonia and bilirubin, was seen in most clinical studies. Average ammonia and total bilirubin concentrations were not reported in the TECA-HALSS and MELS studies. No improvement in ammonia concentrations was reported in the LSS study. According to Mundt et al,[86] the assessment of only biochemical variables before and after liver support treatment might fail to detect a beneficial effect, because of continuing deterioration of the patient. Ellis et al[61], Hughes and Williams,[121] and Colletti et al[122] emphasized that any additional function provided by the device is difficult to assess because changes in blood tests may not discriminate between synthetic/detoxification functions of the liver assist device and those of the native liver. Comparing plasma samples from the inlet and outlet of the device at the same time can also be used to assess efficacy of the BAL treatment. Except for the ELAD system, which was associated with an increase in ammonia and bilirubin levels, application of other systems (HepatAssist, BLSS, RFB, AMC-BAL) was associated with more or less a biochemical improvement. Clinical application of the AMC-BAL was associated with the largest reduction in average ammonia and total bilirubin levels (Table 4). However, the variation between patients and duration of BAL treatments may have influenced this outcome.

Adverse Events

Adverse events were only reported for 4 BAL systems (i.e., ELAD, HepatAssist, BLSS, AMC-BAL). In these systems, transient hypotension was occasionally observed at the beginning of BAL treatment. After treatment with fluid expansion and/or dopamine, the hypotension associated with the BLSS and AMC-BAL systems was readily reverted and hemodynamics stabilized. In the HepatAssist trials, 1 patient with hypotension was reported. In this case, BAL treatment was immediately discontinued. This episode of hypotension might have been caused by pre-existing hypovolemia, by bradykinin release as a reaction to the extracorporeal circuit, or by xenogeneic antigens. The release of bradykinin has previously been associated with blood contacting artificial membranes or plasma filters. Along the same lines, bradykinin has been shown to cause a drop in mean arterial pressure within 10 minutes after the start of continuous renal dialysis.[123] Except for this transient hypotension, no other important BAL-related adverse events have been reported. In addition, no clinically manifest adverse immunologic reactions have been observed during short-term treatment with BAL systems charged with allogeneic or xenogeneic hepatocytes.

CONCLUSIONS

The concept of BAL support has proven to be successful in animal studies. In addition, clinical application of BAL devices has proven safe. Clinical assessment of BAL treatment is severely hindered by the variation in the patient groups studied and the fact that most patients undergo sub-

sequent OLT. However, neurologic and biochemical parameters improved after treatment with different BAL systems. To ultimately determine the effect of BAL treatment on survival, controlled, randomized clinical trials in large patient groups are required to yield statistically significant outcomes. In parallel, BAL research should focus on the replacement of hepatocytes of animal origin by hepatocytes of human origin, either primary hepatocytes or immortalized cell lines, to overcome possible immunologic reactions and zoonosis.

ACKNOWLEDGMENTS

The authors thank Dr. F. Calise, head of Centro di Biotecnologie and Liver Transplantation Unit Cardarelli Hospital, Naples, Italy, and his coworkers for their contribution to the phase I clinical trial of the AMC-BAL. The development of the AMC-BAL was partly granted by Technology Foundation STW, Utrecht, The Netherlands.

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