Int J Artif Organs 2012; 35 (7): 503-510 DOI: 10.5301/ijao.5000099

ORIGINAL ARTICLE

Artificial liver support system reduces intracranial pressure more effectively than bioartificial system: an experimental study

Ondrej Ryska[1], Tomas Pantoflicek[2,3], Eva Laszikova[3,4], Josef Prazak[5], Eva Koblihova[2], Miroslav Ryska[2,3]

[1] Surgery Department, First Faculty of Medicine, Charles University, Faculty Hospital, Bulovka, Prague - Czech Republic
[2] Surgery Department, Second Faculty of Medicine, Charles University and Central Military Hospital, Prague - Czech Republic
[3] Centre for Cell and Tissue Therapy, Second Faculty of Medicine, Charles University, Prague - Czech Republic
[4] Department of Anaesthesiology, Central Military Hospital, Prague - Czech Republic
[5] Department of Anaesthesiology, Resuscitation and Intensive Care, Second Faculty of Medicine, Charles University, Prague - Czech Republic

ABSTRACT

Objectives: *Extracorporeal liver support (ELS) may play a role in bridging therapy in patients with acute liver failure (ALF). The aim of this study was to compare the influence of nonbiological and biological methods on intracranial pressure (ICP) in an animal model of ALF.*

Methods: *A surgical devascularization model of ALF in pigs (35-40 kg) was used. Elimination therapy started after the onset of hypoglycemia. Biochemical parameters (bilirubin, ammonia, lactate, etc.) as well as ICP and cerebral perfusion pressure (CPP) were monitored for 12 hours. Of the total 31 pigs with ALF, 14 animals were treated by fractionated plasma separation and absorption (FPSA), 10 were treated with a bioartificial liver (BAL), and 7 animals were used as a control group.*

Results: *FPSA and BAL treatment started on average 3 hours 17 minutes and 2 hours 21 minutes, after devascularization and lasted for 5 hours 54 minutes and 5 hours 43 minutes, respectively. Ammonia levels were lower in the FPSA group, and bilirubin levels differed significantly in both the FPSA and BAL groups compared with controls. However, ICP values were reduced more effectively in pigs treated by FPSA: 19.1 vs. 27.0 mm Hg at 9 hours, 22.5 vs. 28.7 mm Hg at 11 hours, and 24.0 vs. 33.0 mm Hg at 12 hours ($p<0.05$).*

Conclusions: *The artificial liver support system FPSA reduced ICP values more effectively than the Performer O. Liver RanD BAL system. Compared with this BAL system, the nonbiological elimination method of FPSA is a simpler application with the advantage that it can be applied in a more continuous way.*

KEY WORDS: *Acute liver failure, Bioartificial liver support system, Fractionated plasma separation and absorption, Intracranial pressure*

Accepted: February 8, 2012

INTRODUCTION

Acute liver failure (ALF) is a clinical status defined as a sudden loss of liver function, followed by hepatic encephalopathy (HE) and coagulopathy in a patient without previous liver disease (1). The mortality rate of ALF patients in the pretransplant era reached 80%-85% (2). With the development of modern intensive care, the overall mortality remains in between 20% and 40%. Approximately 25%-45% of ALF patients are therefore candidates for

acute liver transplantation (LT) (2, 3). Acute LT is the only method of improving survival, but is limited by a shortage of organ donors, therefore some patients die on the waiting list. Spontaneous liver recovery is achieved in about 40% of cases (3).

Extracorporeal liver support (ELS) can possibly help to bridge this life-threatening period. Artificial systems are based on removing toxins through hemodialysis, hemofiltration, or by absorption of albumin-bound substances. Bioartificial support systems using living hepatocytes can possibly combine the synthetic functions with effective detoxification. Despite intensive development, these methods have not yet been established in clinical practice, and their benefits, as assessed by biochemical markers as well as in improving the survival rate of ALF patients, are still under discussion (4).

Intracranial hypertension and development of HE is one of the most important negative prognostic factors in ALF. Therefore, effective ELS should prevent the rapid increase of intracranial pressure (ICP) (5).

The aim of our study was to compare values of ICP during bridging therapy by means of nonbiological or biological support systems in an experimental model of ALF.

METHODS

A surgical model of ALF in pigs weighing 35-40 kg was used. ALF was induced surgically in 31 animals. A total of 14 animals were treated with fractionated plasma separation and adsorption (FPSA; Prometheus, Fressenius, Germany) and 10 animals with a bioartificial liver (BAL; O. liver Performer, Rand, Italy). The remaining 7 animals were used as an untreated control group.

The onset of ALF was determined by hypoglycemia (<3.5 mmol/L). The experiment was stopped 12 hours after the onset of ALF.

Anesthesia

Premedication with an intramuscular injection of a mixture comprising 10 mg/kg ketamine (Narkamon; Leciva, Czech Republic), 0.2 mg atropine (Atropin Biotika; Hoechst-Biotika, Slovakia), 4 mg/kg azaperone (Stresnil; Janssen Pharmaceutica, Belgium), and 25 µg/kg medetomidine hydrochloride (Domitor; Pfizer, USA) was given to the animals 20 minutes before surgery.

General anesthesia was induced with an intravenous injection of a mixture comprising 4 µg/kg fentanyl (Fentanyl Torrex; Torrex Chiesi, Austria) and 0.3 mg/kg etomidate (Hypnomidate; Janssen Pharmaceutica, Belgium); animals were then intubated, and ventilation was performed with a Siemens Servo 900 C ventilator (Siemens, Elema, Sweden) using pressure-controlled ventilation with $Fi0_2$ 0.4, positive end-expiratory pressure (PEEP) 4, F16/minute, 6 to 8 ml/kg tidal volume, and normocapnia was maintained ($paCO_2$ 4.6-5.3 kPa). During general anesthesia, inhalation of isoflurane (Forane; Abbott Laboratories, UK) mixed with air and a continual infusion of a combination of 6-10 µg/kg fentanyl (Fentanyl Torrex; Torrex Chiesi, Austria) and 1 µg/kg per minute medetomidine hydrochloride (Domitor; Pfizer, USA) was applied. Animals were also given boluses of 0.02 mg/kg pipecuronium bromide (Arduan; Gedeon Richter, Hungary) during the surgical operation. Amoxicillin 1.2 g and quamatel 1 amp were used as antibiotic and stress ulcer prophylaxes. Using the puncture method, the femoral vein and artery were cannulated, which was necessary to connect the animals into the ELS device and to provide invasive blood pressure monitoring. The internal jugular vein was detected using surgical venesection, and a thermodilution pulmonary artery catheter (7F Arrow, USA) was inserted to monitor hemodynamic parameters.

Devascularization

ALF was induced by surgical devascularization of the liver. The abdominal cavity was opened through a midline laparotomy, and a self-retaining retractor was inserted. An epicystostomy and gastrostomy were constructed. The portal vein was dissected, and a portocaval end-to-side anastomosis (PCA) was performed using a continuous pattern of 5/0 Prolene sutures. The common bile duct and hepatic artery were identified and tied off. When completed, hemostasis was achieved, and the abdomen was closed. This model was evaluated by authors previously in the sham-controlled study including the histological confirmation of liver ischemia (6).

Postsurgical care and monitoring

After surgery, each animal was placed on its right side and sedated using drugs with an extrahepatic elimination pathway. An ICP intraparenchymal sensor (Codman; Johnson & Johnson, USA) was inserted into the right cerebral

hemisphere to provide ICP monitoring. Cerebral perfusion pressure (CPP) was calculated from the mean arterial pressure, MAP (CPP = MAP–ICP), which was maintained at a level higher than 60 mm Hg, by continuously infusing crystalloid and colloidal solutions. When ineffective, a continuous infusion of noradrenaline (Noradrenalin; Leciva, Czech Republic) was used. Blood glucose levels <3.5 mmol/L were considered as a clinical sign of ALF and were treated using continuous infusion of 40% glucose solution, to reach a normal blood glucose concentration (3.5-5.0 mmol/L). The animal was warmed up with warming pads during the whole experiment to avoid hypothermia. Blood samples were taken for blood gas analysis, hemoglobin levels, hemocoagulation parameters, and biochemical parameters (liver function tests as well as creatinine, urea, bilirubin, and ammonia levels).

Isolation of hepatocytes, filling and connection of bioreactor

Two-phase collagenase (Collagenase Cruda activity 1,000) liver perfusion with stabilization by Krebs-Henseleit medium and subsequent centrifugation at 500 rpm was selected as a method for hepatocyte isolation (7). Whole warm ischemia did not exceed 55 minutes. By this procedure, 80 g of a cellular suspension was obtained and diluted in a total volume of 200 mL of Krebs-Henseleit medium. Cell count was 7×109-10×109, cells and viability was assessed by methylene blue (85% on average). Next, the suspension of isolated hepatocytes was placed into the bioreactor, which was stored for 4-6 hours before use in a thermo box at 4°C. The radial flow bioreactor (O. liver cart-A; RanD S.r.l., Cavezzo, MO, Italy), developed specifically for liver cells, was used in the study. The plasma diffuses from inside to outside the bioreactor, after passing through the compartment containing the hepatocytes seeded in the 3-dimensional structure. This radial flow allows direct contact between cells and plasma. The porosity of the polyester membrane (1 μm) allows a broad exchange of solutes and molecules but not the escape of liver cells into the circulation.

The bioreactor was connected to the O. liver BAL extracorporeal system (tubing volume 180 mL), which consists of 2 independent circuits. In the first circuit, plasma is separated in the plasma filter and collected in the 2-L reservoir (at speed of 80-100 ml/min). During the therapy, separated plasma is pumped from reservoir to the second circuit which allows circulation through the oxygenator (mixture composed of 95% O_2 and 5% CO_2), the cartridge of synthetic resin used for bilirubin and bile acid absorption, and the bioreactor with hepatocytes (speed of bioreactor circuit was 200-250 ml/min). The viability of the cells seeded in the bioreactor during the therapy was confirmed by continual measurement of glucose consumption calculated as the difference in glucose levels at the inlet and outlet of the bioreactor.

An anticoagulation system with citrate/calcium was used during the therapy, which was performed for 6 hours.

FPSA system

The blood pump of the Prometheus system conducted the animal's whole blood into an AlbuFlow filter where plasma was separated, including the albumin fraction. Plasma was then conducted through the secondary circuit into 2 adsorbers (Prometh1 and Prometh2). The speed of the plasma circuit pump was always about 60% higher than that of the blood pump, which allowed more effective plasma purification in the secondary circuit. Subsequently, the whole blood was conducted into a dialyzer (HiFlux), cleaned, and returned to the animal's body through the left femoral vein. The whole tubing volume of the system was 200 mL. During the 6 hours of FPSA therapy, the citrate/calcium system was used as an anticoagulant.

At the end of our experiment, animals were sacrificed by a lethal dose of thiopental and potassium chloride solution. Necropsies were then performed with abdominal viscera visualization.

Control group

Seven animals in which the devascularization was performed were included in the control group. These pigs had identical invasions (pulmonary artery catheter, femoral artery and vein catheter) and monitoring. After the onset of hypoglycemia they were treated only with standard medical therapy for volume therapy and glucose infusion to keep blood glucose levels within the physiological range (3.5-5.0 mmol/L). The infusion of noradrenalin was maintained to reach a MAP above 60 mm Hg, when volume therapy only was insufficient, according to the same protocol used in both interventional groups.

Statistical analysis

The laboratory parameters were compared within the FPSA, BAL, and control groups. For the purpose of descriptive statistics, data are presented as median values with appropriate percentiles or as means ± standard deviation. For statistical analysis of our data we used MedCalc 11.4.4.0. software. Bartlett's test, Student's *t*-test, and the Mann-Whitney *U*-test were used. Statistical significance was defined as a p value of less than 0.05.

Declaration

Preoperative treatment, surgical operations, and postoperative care were undertaken according to the protection against cruelty to animals, law no. 312/2008 Coll., and the protection, breeding, and the use of experimental animal decree no. 207/2004 Coll. The experiment protocol was approved by the expert and ethics committees of the Institute of Clinical and Experimental Medicine in Prague.

RESULTS

in A total of 38 animals were included in experimental study. FPSA therapy was performed in a group of 17 animals, 3 pigs were excluded from the study: 1 due to technical problems during the extracorporeal perfusion (system clotting) and 2 animals because of intracranial bleeding after ICP sensor placement. The BAL system was applied to 14 animals. Four pigs were excluded from the study: 1 because of severe bleeding as a complication of surgical procedure, 2 due to primary constrictive pericarditis (verified by autopsy) followed by cardiopulmonary failure during the experiment, and 1 animal because of protocol deviation by hydrocortisone administration.

The onset of hypoglycemia was observed after 3 hours and 17 minutes (range 2:15-4:20) after devascularization in the FPSA group. FPSA therapy was administered for 5 hours and 54 minutes (range 5:45-6:00) on average. In the BAL group, hypoglycemia occurred after 2 hours and 21 minutes (range 1:00-3:45), and therapy took place for 5 hours and 43 minutes (range 3:25-8:10) on average. There was no significant difference in the time of hypoglycemia onset among the FPSA, BAL, and control groups, where it took 2 hours and 55 minutes (range 1:45-3:20) (p>0.05).

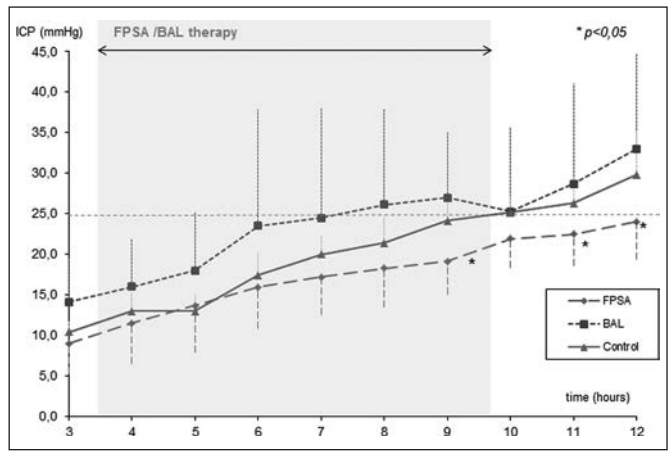

Fig. 1 - *Intracranial pressure (ICP; mm Hg) values during the experiment. *p<0.05, significant decrease in fractionated plasma separation and adsorption (FPSA) group vs. bioartificial liver (BAL) and control groups.*

The ICPs measured from the third hour of the experiment is summarized in Figure 1.

Significantly lower ICP levels were observed at hour 9 (19.1 ± 4.1 mm Hg vs. 27.0 ± 8.0 mm Hg; p = 0.004), 11 (22.5 ± 4.0 mm Hg vs. 28.7 ± 10.2 mm Hg; p = 0.04), and 12 (24.0 ± 4.7 mm Hg vs. 33.0 ± 11.7 mm Hg; p = 0.02) in the FPSA group, in comparison with the BAL group. The ICP from the ninth hour in the control group was significantly higher compared with that of the FPSA group: hour 9 (19.1 ± 4.1 mm Hg vs. 24.1 ± 2.8 mm Hg; p = 0.0095), 10 (21.9 ± 3.6 mm Hg vs. 25.1 ± 2.1 mm Hg; p = 0.04), 11 (22.5 ± 4.0 mm Hg vs. 26.3 ± 3.5 mm Hg; p = 0.04), and 12 (24.0 ± 4.7 mm Hg vs. 29.8 ± 5.8 mm Hg; p = 0,02). The absolute increase of ICP between the third and 12th hour was also higher in the BAL group compared with the FPSA group (15.0 mm Hg vs. 18.9 mm Hg; p = 0.04).

A significant difference in CPP values was also observed in the FPSA group compared with the control group (Fig. 2).

Furthermore, a significantly (p<0.05) lower level in ammonia was seen in the last 3 hours of the experiment after FPSA therapy compared with the untreated control group. Differences in this parameter between the FPSA and BAL groups were not significant (Tab. I).

Bilirubin levels were significantly lower in both the FPSA and BAL groups (p<0.05) in comparison with the control group after the sixth hour of the study (Tab. II).

There were no significant differences between all groups observed for other biochemical markers (urea, creatinine,

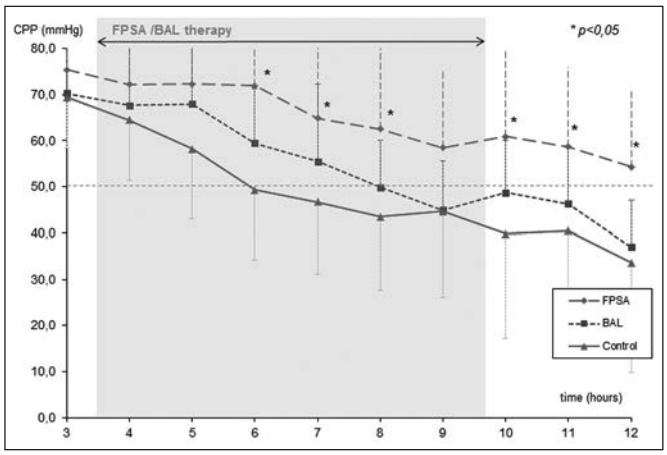

Fig. 2 - *Cerebral perfusion pressure (CPP; mm Hg) values during the experiment. *p<0.05, significant increase in fractionated plasma separation and adsorption (FPSA) group vs. bioartificial liver (BAL) and control groups.*

aspartate transaminase, alanine transaminase, Gamma-glutamyl transpherase, alkaline phosphatase, lactate, or blood gas analysis).

DISCUSSION

The liver plays a central role not only in detoxification but also in synthetic pathways, and contributes to more than 500 different metabolic processes (3). Potentially effective elimination methods must therefore be able to eliminate circulating toxins and also to replace the synthetic liver functions and prevent the development of infection (8).

ELS has been the topic of continued experimental and clinical evaluation for the last 40 years. The BAL systems containing hepatocytes have the theoretical advantage of

TABLE I - VALUES OF AMMONIA (μMOL/L) DURING COURSE OF EXPERIMENT

Group	Time (hours)	0	3	6	9	12
FPSA (n = 14)	Mean (SD)	59.3 (38.3)	249.6 (73.8)	258.2 (62.7)	274.9 (91.8)*	391.4 (92.9)*
	(95% CI)	(39.2-79.3)	(210.9-288.3)	(225.3-291)	(226.8-323)	(340.9-441.9)
BAL (n = 10)	Mean (SD)	49.6 (21.1)	212.2 (97.1)	288.0 (93.5)	390.0 (213)	480.6 (206.4)
	(95% CI)	(36.5-62.7)	(152.1-272.4)	(226.9-349.2)	(258-522)	(299.6-661.5)
Control (n = 7)	Mean (SD)	39.2 (14.8)	212.4 (93.2)	305.9 (121.8)	414.3 (246.4)	592.1 (387.5)
	(95% CI)	(28.9-49.4)	(147.9-277)	(221.5-390.3)	(243.5-585)	(323.6-860.7)

BAL = bioartificial liver; CI = confidence interval; FPSA = fractionated plasma separation and adsorption.
*p<0.05, vs. controls.

TABLE II - LEVELS OF BILIRUBIN (μMOL/L) DURING COURSE OF EXPERIMENT

Group	Time (hours)	0	3	6	9	12
FPSA (n = 14)	Mean (SD)	5.4 (3.7)	15.4 (7.4)	12.8 (6.5)*	11.9 (4.1)*	13.9 (6.3)*
	(95% CI)	(3.5-7.4)	(11.5-19.3)	(9.4-16.2)	(9.8-14.1)	(10.4-17.3)
BAL (n = 10)	Mean (SD)	4 (1.4)	16.4 (6.8)	10.6 (3.2)*	11.8 (4.4)*	12.9 (4.6)*
	(95% CI)	(3.2-4.9)	(12.2-20.6)	(8.5-12.7)	(9-14.5)	(8.9-16.9)
Control (n = 7)	Mean (SD)	5.7 (4.4)	22.7 (11.1)	29.8 (10)	30 (12.4)	26.1 (12.2)
	(95% CI)	(2.6-8.8)	(15-30.4)	(22.9-36.8)	(21.4-38.5)	(17.6-34.6)

BAL = bioartificial liver; CI = confidence interval; FPSA = fractionated plasma separation and adsorption.
*p<0.05, vs. controls.

replacing both detoxification and synthetic liver functions (protein synthesis and gluconeogenesis). Experimental studies evaluating BAL, performed at the beginning of the century, brought very promising results. Flendrig et al confirmed in an experimental study, the survival benefit of BAL using fresh porcine hepatocytes by pigs with ALF induced by devascularization (9). Also a minor effect on intracranial hypertension was observed in a survival study in a porcine ischemic ALF model including 18 animals, which was published in 2001 (10). Another study from Japan compared artificial support therapy presented by continuous hemodiafiltration and plasma exchange with BAL equipped by a hollow fiber bioreactor seeded by porcine hepatocytes. This study concluded that both methods prolonged survival, but the artificial methods had major effects on toxin elimination (bilirubin and ammonia detoxification). ICP was unfortunately not evaluated (11).

However, these results have been difficult to repeat in clinical trials later. Five manufactured devices similar to that used in the present study have been clinically tested (HepatAssist, ELAD, MELS, BLSS, and AMC-BAL), but only 2 were evaluated in randomized trials. The largest study performed on the HepatAssist included 171 patients with ALF. Although there was a positive effect on ammonia and bilirubin levels, 30-day survival remained unchanged (12).

Of different examined types of artificial systems, the molecular absorbent recycling system (MARS) (13) and FPSA have been used most commonly in previous studies (14). Prospective randomized evaluation has been performed with both systems on patients with ALF. A significant effect of MARS was demonstrated on several biochemical markers (bilirubin, ammonia, etc.), but the survival rate did not increase (15). During the largest promising randomized trial with FPSA (HELIOS), 145 patients were randomized to groups treated with standard medical therapy (SMT) and SMT + Prometheus. FPSA therapy did not significantly influence overall mortality (at 28 and 90 days) (16). However, comparative studies have shown a higher efficiency of FPSA in eliminating low-molecular-weight and albumin-bound substances in comparison with MARS (17).

A recent meta-analysis summarized the results of 8 randomized trials evaluating biological and nonbiological liver support (197 patients with ALF and 157 patients with acute-on-chronic liver failure) and concluded that ELS systems may improve survival in ALF. There is no evidence that they improve survival in acute-on-chronic failure (18).

Some studies support the theory that the discontinuation of elimination, which will again lead to a deterioration of laboratory markers and the patient's condition, may compromise the overall results (19). Regarding this fact, a survival study should not be performed unless ELS is used continuously, which is nearly impossible in an experimental study. The aim of our study was to evaluate the effect of only 1 treatment cycle with FPSA or BAL therapy under standardized conditions using a relevant surgical model of ALF (20, 21).

HE is one of the most important prognostic factors in ALF. It is defined as a number of reversible neurological and psychological defects accompanying loss of liver function (22). The "multiple-hit hypothesis" explains the higher permeability of the blood–brain barrier because of astrocyte swelling caused by increased ammonia level. This leads to an influx of proinflammatory endotoxins which deteriorate cerebral hemodynamics and increase the ICP (23). Even if intensive care is maintained, malignant intracranial hypertension may begin and progress to death (5). ELS can prevent the development of HE; first, due to an active reduction of serum ammonia, and second by reducing levels of proinflammatory cytokines which have already been shown in previous studies with FPSA (24).

In the group treated by FPSA in our study, the ammonia level was stabilized during the first third of therapy, followed by stabilization of ICP. Mean ICP in animals treated with FPSA was significantly lower than in the control group after 9 hours and did not reach a critical level (over 25 mm Hg), when irreversible changes in the cerebral parenchyma can be caused (25). On the other hand, in the group treated by BAL, the increase in ICP progressed until the last third of therapy. As a logical consequence, the CPP was significantly higher during and after FPSA therapy and did not drop below 50 mm Hg (limit of hypoperfusion causing irreversible changes) (26) in contrast to the BAL and control groups. The initial ICP levels were a little bit higher in the BAL group as a consequence of short-term hemodynamic instability caused by faster onset of hypoglycemia in the BAL group, but the difference was not significant ($p = 0.095$).

The bilirubin levels were significantly lower in both interventional groups after the sixth hour of the experiment. The real outcome of normobilirubinemia is controversial. Bilirubin at higher levels is cytotoxic especially for neurons and cerebral endothelial cells. That is why the decrease of its level is desirable during ALF therapy. On the

other hand, the potential antiapoptotic effect of bilirubin on hepatocytes has been described, which means that hyperbilirubinemia may have a protective role in liver disease (27). The overall failure of BAL therapy can be explained by several factors. One of these is the absence of a source of a sufficient number of vital liver cells, which would present a specific liver function. A study of major liver resection has suggested that 30% of the liver parenchyma is adequate, which corresponds to approximately 450 g of living hepatocytes (28). The commercially available bioreactor used in our study did not have such a capacity. Moreover, the 6 hours of BAL therapy used in our study led to extreme stress to the hepatocytes in plasma with high concentration of toxins, e.g., ammonia, lactate, or aromatic amino acids. This fact caused a rapid decline in hepatocyte viability (29).

CONCLUSIONS

We can conclude that an artificial liver support system is significantly more efficient than a bioartificial one. Results of our experimental study have proven the effectiveness of the artificial liver support system. The improvement of intracranial pressure as well as in the decrease of ammonia levels was found to be significantly higher compared with the bioartificial method. Both support systems effectively reduced bilirubin levels. Bioartificial systems continue to face a number of unsolved issues such as bioreactor capacity. Nonbiological methods are, however, very simple, affordable, and offer the theoretical possibility of continuous therapy.

ABBREVIATIONS

ALF	acute liver failure
BAL	bioartificial liver
CPP	cerebral perfusion pressure
ELS	extracorporeal liver support
FPSA	fractionated plasma separation and adsorption
HE	hepatic encephalopathy
ICP	intracranial pressure
MARS	molecular adsorbent recycling system
PEEP	positive end-expiratory pressure
PERV	porcine endogenous retrovirus

Informed Consent: The experiment protocol was approved by the expert and ethics committees of the Institute of Clinical and Experimental Medicine in Prague.

Financial Support: Financial support was received from the Research Centre, Prague (no. LN00A065), and the Internal Grant Agency, Czech Ministry of Health (nos. NT 11463 and S8120).

Conflict of Interest Statement: Authors disclose any conflict of interest.

Meeting Presentation: This paper was originally presented at the 36th annual meeting of the European Society for Artificial Organs (ESAO 2009), Compiègne, France, September 2-5, 2009.

Address for correspondence:
Ondrej Ryska, MD
Biskupcova 89
130 00 Prague 3, Czech Republic
e-mail: ondrejryska@centrum.cz

REFERENCES

1. Larsen FS, Bjerring PN. Acute liver failure. Curr Opin Crit Care. 2011;17(2):160-164.
2. Hoofnagle JH, Carithers RL, Shapiro C, Ascher N. Fulminant hepatic failure: summary of a workshop. Hepatology. 1995; 21:240-252.
3. Riordan SM, Williams R. Perspectives on liver failure: past and future. Semin Liver Dis. 2008;28:137-141.
4. Liu JP, Gluud LL, Als-Nielsen B, Gluud C. Artificial and bioartificial support systems for liver failure. Cochrane Database Syst Rev. 2009;1:CD003628.
5. Aggarwal S, Obrist W, Yonas H, et al. Cerebral hemodynamic and metabolic profiles in fulminant hepatic failure: relationship to outcome. Liver Transpl. 2005;11:1353-1360.
6. Ryska M, Kieslichová E, Pantoflícek T, et al. Devascularization surgical model of acute liver failure in minipigs. Eur Surg Res. 2004;36:179-184.
7. Selen, PO. Preparation of isolated rat liver cells. Methods Cell Biol. 1976;13:29-83.
8. Rozga J. Liver support technology: an update. Xenotransplantation. 2006;13:380-389.
9. Flendrig LM, Calise F, Di Florio E, et al. Significantly improved survival time in pigs with complete liver ischemia treated with a novel bioartificial liver. Int J Artif Organs. 1999; 22(10):701-709.
10. Khalili TM, Navarro A, Ting P, et al. Bioartificial liver treatment prolongs survival and lowers intracranial pressure in pigs with fulminant hepatic failure. Int J Artif Organs. 2001;25(7): 566-570.

11. Kawazoe Y, Eguchi S, Sugiyama N, Kamohara Y, Fujioka H, Kanematsu T. Comparison between bioartificial and artificial liver for the treatment of acute liver failure in pigs. World J Gastroenterol. 2006;12(46):7503-7507.
12. Demetriou AA, Brown RS Jr, Busuttil RW, et al. Prospective, randomized, multicenter, controlled trial of a bioartificial liver in treating acute liver failure. Ann Surg. 2004;239:660-667.
13. Stange J, Mitzner SR, Klammt S, et al. Liver support by extracorporeal blood purification: a clinical observation. Liver Transpl. 2000;6:603-613.
14. Hassanein TI, Tofteng F, Brown RS Jr, et al. Randomized controlled study of extracorporeal albumin dialysis for hepatic encephalopathy in advanced cirrhosis. Hepatology. 2007;46:1853-1862.
15. Rifai K, Kribben A, Gerken G, et al. Extracorporeal liver support by fractionated plasma separation and absorption (Prometheus®) in patients with acute-on-chronic liver failure (HELIOS study): a prospective randomized controlled multicenter study. J Hepatol. 2010;52:S3.
16. Laleman W, Wilmer A, Evenepoel P, et al. Effect of the molecular adsorbent recirculating system and Prometheus devices on systemic haemodynamics and vasoactive agents in patients with acute-on-chronic alcoholic liver failure. Crit Care. 2006;10(4);R108.
17. Stutchfield BM, Simpson K, Wigmore SJ. Systematic review and meta-analysis of survival following extracorporeal liver support. Br J Surg. 2011;98:623-631.
18. Sussman NL, McGuire BM, Kelly JH, et al. Hepatic assist device: will they ever be successful? Curr Gastroenterol Rep. 2009;11:64-68.
19. Terblanche J, Hickman R. Animal models of fulminant hepatic failure. Dig Dis Sci. 1991;36:770-774.
20. Lee WM, Squires RH Jr, Nyberg SL, et al. Acute liver failure: summary of a workshop. Hepatology. 2008;47: 1401-1415.
21. Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy: definition, nomenclature, diagnosis and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. Hepatology. 2002;35:716-721.
22. Wright G, Jalan R. Ammonia and inflammation in the pathogenesis of hepatic encephalopathy. Pandora's box? Hematology. 2007;46:291-294.
23. Santoro A, Faenza S, Mancini E, et al. Prometheus system: a technological support in liver failure. Transplant Proc. 2006;38:1078-1082.
24. Steiner LA, Andrews PJ. Monitoring the injured brain: ICP and CBF. Br J Anaesth. 2006;97:26-38.
25. Asgeirsson B, Grande PO, Nordstrom CH. A new therapy of post-trauma brain oedema based on haemodynamic principles for brain volume regulation. Intensive Care Med. 1994;20:260-267.
26. Granato A, Gores G, Vilei MT, Tolando R, Ferraresso C, Muraca M. Bilirubin inhibits bile acid induced apoptosis in rat hepatocytes. Gut. 2003;52(12):1774-1778.
27. Strain AJ, Neuberger JM. A bioartificial liver: state of the art. Science. 2002;295:1005-1009.
28. Papadimitriou D, Arkadopoulos N, Kouskouni E, et al. Hepatocyte function during experimental use of a bioartificial liver. Transplant Proc. 2004;36:1741-1743.