Temporary Liver Support: Current Concepts

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ABSTRACT

The occurrence of end-organ dysfunction in patients with liver injury defines a poor prognosis. The use of temporary liver support is based upon the concept that the liver has the capacity to regenerate and recover if the function of the liver can be maintained during the episodes of acute decompensation. Current research focuses upon how a precipitant such as an episode of gastrointestinal bleeding or sepsis may start a cascade of events that culminate in end-organ dysfunction and liver failure. Our current strategy for the management of liver failure involves supportive therapy for the end-organs with the hope that the liver function would recover if sufficient time for such a recovery is allowed. Because, liver failure, whether of the acute or acute-on-chronic variety, is potentially reversible, the stage is set for the application of newer liver support strategies to enhance the recovery process. Two approaches of liver support have been developed. The first is based around the use of hepatocytes in an extracorporeal device. Although this kind of approach is exciting, current data do not support its widespread usage due to a vast variety of problems such as lack of convincing data, logistic difficulties of actually using the available devices, astronomical cost and the fear of xenozoonoses. Devices based upon the principles of albumin dialysis such as the molecular adsorbents recirculating system (MARS) hold enormous potential. Early data suggest that MARS favourably impacts upon some of pathophysiological factors that are thought to be important in the development of liver failure and possibly improved survival. Large scale clinical trials are underway to further define its exact place in the management of liver failure.

Liver failure occurs either as acute liver failure (ALF) developing *de novo* or as acute decompensation of chronic liver disease (ACLF). The clinical manifestations are fairly similar characterised by multiorgan dysfunction characterised by encephalopathy (HE), hypotension, and renal failure (HRS).[1,2] Unlike chronic decompensation of end-stage liver disease, ALF and ACLF are potentially reversible. A supportive therapy which can tide over the acute period of crisis (and act as a bridge to liver transplantation in cases of ALF) can be possibly life-saving.[3] This essential premise of possible reversibility has lead to the attempts over the past 40 years to develop an extracorporeal liver support system. Essentially, two types of liver support systems are under development. (A) Bio-artificial devices, using hepatocytes is based upon the concept that they can perform the functions of the failing liver and (B) Artificial devices, of which the one currently being tried extensively is the Molecular Adsorbents Recirculating System (MARS), utilising the principles of albumin dialysis.

BIOARTIFICIAL LIVER SUPPORT DEVICES

This form of liver support system has two components, a bio-reactor and hepatocytes. Bioreactor is device which houses the hepatocytes allowing free exchange of molecules between plasma/blood and hepatocytes across a membrane. The membrane allows movement of 'toxins' as well as transport proteins like albumin, while preventing passage of immunoglobulins, complements or viruses and cells. The hepatocytes use oxygen and nutrients and detoxify toxins from the plasma. The metabolites thus generated are passed back into the plasma.[4]

Theoretically, viable hepatocytes in these systems should therefore reproduce the synthetic, detoxifying as well as excretory functions of the liver. Clearly, the best type of cells to use would be human liver cells but the supply of such cells is limited and they are difficult to grow in culture.[4] Two alternative approaches have been tried; (a) an approach is been to produce cells using genetic engineering techniques to provide them with both the ability to grow in culture while maintaining with the desired functional capabilities; (b) another approach is to use primary hepatocytes obtained from a pig. Thus, the C3A hepatocyte line, a sub-clone of the ubiquitous HepG2 hepatoblastoma cell line, has been used in one system (the Extracorporeal Liver Assist Device [ELAD] developed by Sussman and colleagues).[5] However, while these cells survive and replicate adequately, they are functionally not very competent. Another immortalized human hepatocyte cell line under investigation is HHY41, which retains many liver-specific functions, protein synthesis, gluconeogenesis, and cytochrome P450 activity and is particularly resistant to acetaminophen.[6-8] The escape of tumorigenic cells from the human hepatoblastoma cell line is a potential hazard in the

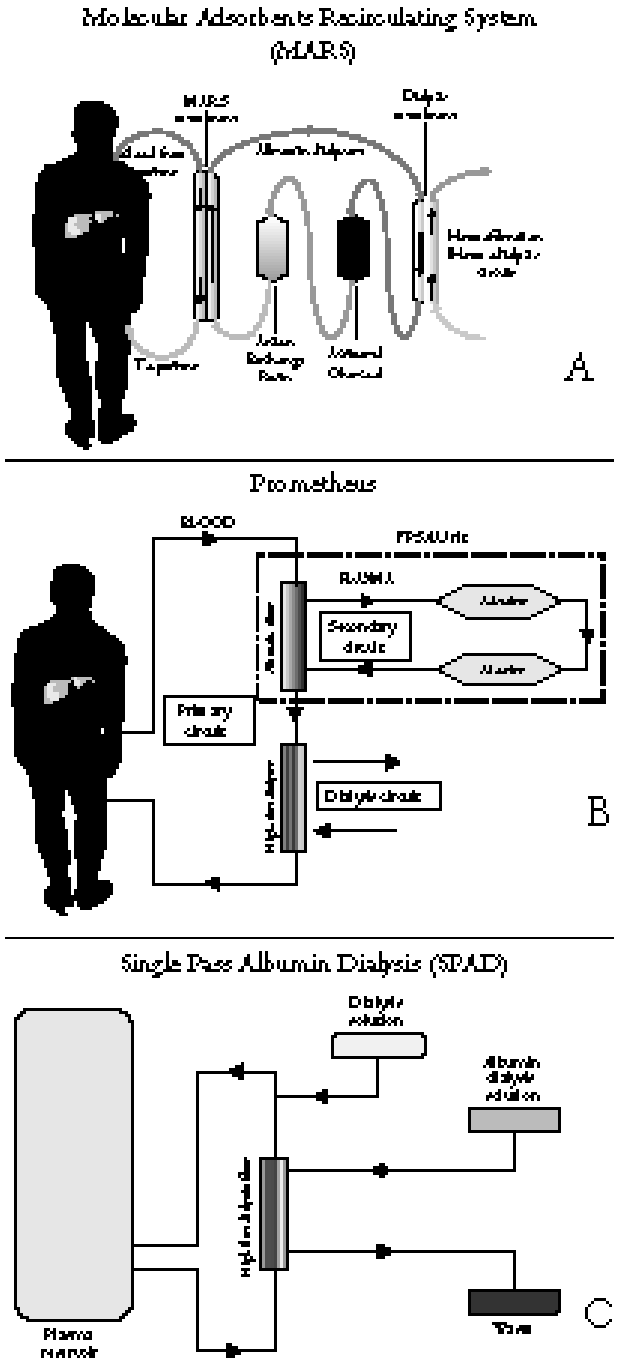

Fig. 1: Schematic representation of the systems utilizing the concept of albumin based detoxification. (a) The molecular adsorbent recirculating system (b) Prometheus (c) Single pass albumin dialysis.

ELAD system. The addition of downstream cell filters to remove immortalized cells from the circulating fluid is generally regarded as being an adequate safety measure against seeding, but safety concerns remain. The second approach has been used in the HepatAssist device developed by Demetriou and colleagues,[9] and the Academic Medical Center -BAL [AMC-BAL] developed by Chamuleau et al.[10] The advantage of porcine hepatocytes, is that they can be satisfactorily cryopreserved, with cell isolation followed by storage at a clinical site prior to use, thereby avoiding the costs and contamination risks of long-term hepatocyte culture.[11] However, as with human tumorigenic cells, safety concerns have been raised regarding the use of porcine cells too, specifically with respect to immune reactions to foreign antigens and xenozoonosis. Porcine endogenous retrovirus (PERV) is ubiquitous among bred pigs, and transmission to humans via BAL has been a persistent fear. PERV DNA and RNA have been detected in the supernatant of pig hepatocyte culture systems.[12] *In vitro* studies have found that these viruses can infect human cell lines.[13-17] However, PERV transmission to humans was not demonstrable in *in vivo* studies.[18-20] A recent study also could not find any evidence of PERV infection, using reverse transcriptase polymerase chain reaction, 6 months after BAL treatment.[21] In any case, these objections have lead to a moratorium against the use of such devices in the UK and most parts of Europe. The future of BAL clearly rests on finding the right hepatocyte.

Results of Clinical Studies using BAL. Data for the most important studies using the bioartificial liver support devices is summarised in Table 1. Similarly, none of the randomized trials evaluating the various BAL systems have demonstrated a clear survival advantage.[22-24] The largest one[22] showed a significant survival benefit only in the acetaminophen subgroup. Thus, while the clinical effects of liver support using hepatocytes have been tantalizingly interesting, a vast amount of work still needs to be carried out before any clear benefit is demonstrated beyond doubt.

EXTRACORPOREAL ALBUMIN DIALYSIS

The mechanisms underlying the development of the multi-organ dysfunction of liver failure are, as yet, poorly understood. The 'toxin hypothesis' implicates a variety of toxins which accumulate as a result of impaired hepatic metabolism/ detoxification. Ammonia, protein breakdown products (aromatic amino acids, tryptophan, indole, mercaptan, phenol) and endogenous benzodiazepines, among others, are implicated in the development of hepatic encephalopathy. Nitric oxide (NO) and prostanoids are believed to be important in the pathogenesis of circulatory and renal dysfunction. Pro-inflammatory cytokines probably have wide-ranging influences, and oxidative stress has effects ranging from increased capillary permeability to modulating cell death.[25] However the vast majority of these toxins (except possibly ammonia) are water-insoluble and albumin-bound, and conventional renal replacement therapy cannot effectively remove them.

Intravenous albumin administration is important in the treatment of patients with cirrhosis,[26-28] and improves survival in those with spontaneous bacterial peritonitis[29] or with hepatorenal syndrome,[30,31] but the benefits exceed what could have been expected if it was acting simply as a volume expander. Some studies suggest that albumin is an important molecule involved in detoxification and binds various substances,[32] and is perhaps more important in liver diseases than was previously thought.[33] This is the basis for the use of albumin as a binding and scavenging molecule in devices based on albumin dialysis, and therefore the trial of such devices in patients with liver failure. There are three extracorporeal systems that are based upon the concept of albumin dialysis in clinical trials/application in patients and their current status will be briefly discussed below (Fig. 1).

Table 1: Summary of controlled studies evaluating the bioartificial devices in acute liver failure (BAL and ELAD)

Details	Study Demetrious *et al.* (2004)[22]	Ellis *et al.* (1996)[23]	Millis *et al.* (2001)[24]
Patient population	ALF (*n* = 147), primary graft non-function (*n* = 24)	ALF (*n* = 24). Grp-I: not fulfilling LTx criteria (*n* = 17) Grp-II: fulfilling LTx criteria (*n* = 7)	ALF (*n* = 24) 19 listed for LTx, five not listed
System used	Hepat-Assist (BAL)	ELAD	ELAD
Study design	Multi-center randomized controlled trial	Single center randomized controlled trial	Randomized controlled phase I trial
End-point	30-day mortality	In-hospital mortality or LTx	30-day mortality
Outcome	30-day survival:	Survival:	Listed for LTx:
	All patients: BAL 71%, controls 62%	Grp-I: ELAD 78%, controls 75%	30-day survival: ELAD 83%, controls 43%
	Subgroups:		LTx received: ELAD 92%, controls 43%
	All ALF: BAL 73%, controls 59% (*P* = 0.1)	Grp-II: ELAD 1/3, controls 1/4	
	ALF due to paracetamol: BAL 70%, controls 37% (*P*<= 0.05)		
Comments	Substantial impact of LTx (54% of all patients)	Survival among controls in Grp-I much higher than anticipated	Not adequately powered to look at outcome

ALF, BAL, bioartificial liver; ELAD, Extracorporeal Liver Assist Device.

The Molecular Adsorbents Recirculating System (MARS)

This device has been the most widely studied. In albumin dialysis, blood is dialysed against an albumin-containing solution across a suitable membrane.[34-36] The albumin-bound toxins are potentially taken up by the binding sites of the dialysate albumin and thus removed from blood. Molecular Adsorbents Recirculating System (MARS) (Teraklin AG, Rostock, Germany)[37-39] is based upon the principle of albumin dialysis and the system consist of three compartments - a blood circuit, an albumin circuit and a renal circuit (haemofiltration/ haemodialysis). Blood flows through a hollow fibre dialysis module, where it is dialysed across an albumin-impregnated high-flux polysulfone dialysis membrane. 20% human albumin in the albumin circuit acts as the dialysate, and this is passed through the dialysate compartment of the blood dialyser. These toxins are picked up by the albumin dialysate, which, in turn, is regenerated by haemofiltration/ haemodialysis, followed by passage through two sequential adsorbent columns (containing activated charcoal and anion exchange resin), which remove most of the water-soluble and albumin-bound toxins and thus cleanse it. Substances with a molecular weight of more than 50 kDa such as essential hormones bound to carrier proteins, growth factors and albumin are not removed from the perfused plasma because of the pore size of the MARS membrane. Early *in vitro* studies showed effective removal of unconjugated bilirubin, drugs with a high protein-binding ratio (sulfobromophthalein, theophylline), and a protein-bound toxin (phenol)[40] with MARS. In a recent study we have shown that toxins/drugs of a very wide range can be removed by albumin dialysis that has the potential to bind to albumin. We reported the efficient clearance of fentanyl, an opioid predominantly bound to α-1-acid glycoprotein.[41]

Acute-on-chronic liver failure

After the initial studies showed that MARS reduced bilirubin and produced substantial reduction in the severity of HE and improvements in the circulatory and renal functions in patients with various forms and severity of liver dysfunction.[42-44] The largest series of patients with ACLF (n=26), with intrahepatic cholestasis (bilirubin level > 20 mg/dL), treated with MARS was reported from Rostock.[45] The series included 10 patients with a United Network Organ Sharing (UNOS) status 2b, all of whom survived, and 16 patients with a UNOS status 2a, of who seven survived. Another study on patients with severe acute alcoholic hepatitis treated with MARS (n=8)[16] showed improvement of 3-month predicted mortality (pre-MARS: 76%, post-MARS: 27%), with 50% of patients (4/8) still surviving at 3 months.

The first randomized trial of MARS evaluated 13 ACLF patients with type-I hepatorenal syndrome who were treated with either MARS (n=8) or standard medical therapy including haemodiafiltration (n=5).[46] The mortality rate was 100% in the group receiving haemodiafiltration at day 7 compared with 62.5% in the MARS group at day 7 and 75% at day 30, respectively (P < 0.01). Mean survival was longer in the MARS group, which was accompanied by a significant decrease in serum bilirubin and creatinine, and increase in serum sodium and prothrombin activity. MAP at the end of treatment was significantly greater in the MARS group. Although urine output did not increase significantly in the MARS group, four of the eight patients showed an increase compared with none of the control group.

The most recent (and largest completed) randomized controlled trial, performed in two centres (Rostock and Essen), included 24 patients with ACLF with marked hyperbilirubinemia (serum bilirubin >20mg/dl [340μmol/L]) who were randomized to receive standard medical therapy alone (n=12) or MARS in addition (n=12).[47] The primary end-point of bilirubin <15 mg/dL for three consecutive days was reached in five of 12 MARS patients and in two of 12 control patients. Compared to controls, bilirubin, bile acids and creatinine decreased and MAP and HE improved in the MARS group. Most importantly, albumin

Table 2: Summary of important studies evaluating the MARS device

Study	Patient population	Study design	End-point	Outcome
Stange et al (2000) [63]	ACLF with intrahepatic cholestasis (n=26)	Prospective case series	In-hospital mortality	UNOS 2a status: 7/16 survived UNOS 2b status: 10/10 survived
Mitzner et al (2000)[54]	Type-I hepatorenal syndrome (n=13)	Randomized controlled	30-day mortality	Mortality: controls- 100% (day-7); MARS- 62.5% (day-7) and 75% (day-30) (p<0.01)
Heemann et al (2002)[55]	ACLF (n=24)	Randomized controlled	*Primary:* reduction of serum bilirubin *Secondary:* in-hospital mortality	Improvement of bilirubin, and 30-day survival with MARS (11/12 vs 6/11 controls, p<0.05)
Jalan et al (2003)[64]	ACLF due to acute alcoholic hepatitis (n=8)	Prospective case series	In-hospital mortality	Improvement of 3-month predicted mortality (pre-MARS: 76%, post-MARS: 27%). 3-month survival: 4/8

dialysis was associated with a significant improvement in 30-day survival (11/12, versus 6/11 in controls Table 2).

Acute liver failure

In the context of ALF, no controlled studies have been performed as yet, which is not surprising considering the difficult nature of this task. Novelli et al[48] from Rome have treated nine cases of fulminant hepatic failure. Three patients survived without requiring transplantation. The remaining six were transplanted, of whom four survived, while two died due to sepsis. The authors have extended the series to 16, in whom they report improvement of serum bilirubin, INR and ammonia as well as neurological status (though outcome is not described).[49] Isoniemi[50] (Helsinki) has reported 26 cases of ALF (13-toxic (including paracetamol), one pregnancy-induced, 12-unknown aetiology) managed with MARS. Twenty of the 26 patients (77%) survived, which is a strikingly high proportion. Native liver recovered in 11 cases, eight of whom had a toxic aetiology. Ten patients were transplanted, of whom nine survived. Haemodynamic and neurological improvements were noted following MARS therapy in most cases. Felldin et al (Gothenberg) describe 10 patients of ALF treated with MARS, of who seven survived. Beneficial effect was most evident in those who received five or more sessions of treatment (4/5 survivors).[51,52] A recent small randomised controlled study in patients with hyperacute liver failure found that a single session of MARS treatment (n=8) improved systemic haemodynamics (mean arterial pressure, systemic vascular resistance and cardiac output) compared to controls (n=5), who had only been mechanically cooled to match the MARS group.[53]

Prometheus

Another recently introduced system (1999) which has been presented as albumin dialysis, but which in reality utilizes somewhat different principles, is the fractionated plasma separation and adsorption (FPSA),[54] based upon the principle of fractionation of the plasma with the subsequent detoxification of the native albumin by adsorption. It uses an albumin-permeable membrane with a cut-off of 250 kDa. Albumin, and possibly other plasma proteins with their bound toxins cross the membrane and pass through special adsorbers (one or two columns in series in the secondary circuit, containing a neutral resin adsorber and an anion exchanger) that remove the toxins. The cleansed albumin is returned to the plasma. The results of Prometheus treatment in 11 patients with ACLF and accompanying renal failure have recently been published.[55] Improvement of serum levels of conjugated bilirubin, bile acids, ammonia, cholinesterase, creatinine, urea and blood pH occurred. A drop in blood pressure in two patients, and uncontrolled bleeding in one patient were the adverse events noted. Another study compared alternating treatments with MARS and Prometheus in five patients with ACLF. Reduction ratios of both bilirubin and urea were more with Prometheus. Their safety profiles were found to be comparable.[56] Prospective controlled trials are planned for the future.

Single Pass Albumin Dialysis (SPAD)

The newly-developed SPAD system dialyses blood/ plasma against a 4.4% solution of albumin, which is disposed of after a single pass. A standard renal replacement therapy machine is used without any additional perfusion pump system, making the equipment required simpler. This fact, and the use of considerably more diluted albumin as the dialysate (4.4%, as opposed to 20% in case of MARS), offsets the cost of not recirculating the dialysate (in contrast to MARS). Continuous veno-venous haemodiafiltration can be undertaken in conjunction as well. *In vitro* studies suggest that its detoxifying capacity is similar to, or even greater than (especially with regard to bilirubin and ammonia clearance) that of MARS.[57] However, clinical studies need to be performed before any conclusions can be drawn.

CONCLUSIONS

Mortality in patients with liver failure remains unacceptably high. Acute decompensation of cirrhosis carries as poor a prognosis as ALF and improvement in the outcome of these patients may be improved with earlier referral and emerging therapies. The bioartificial liver systems have failed to live up to their initial promise and currently cannot be recommended for the treatment of patients outside of carefully controlled clinical trials. Strategies using albumin dialysis are currently the only viable liver support therapy and have been shown to alter some of the pathophysiological mechanisms thought to be

important in the development of liver failure but progressive thrombocytopenia, coagulopathy and uncontrolled sepsis are relative contraindications to the use of MARS. Results of large on-going trials are necessary to define the exact place of these emerging technologies in the management of liver failure.

REFERENCES

1. Jalan R, Williams R. Acute-on-chronic liver failure: Pathophysiological basis of therapeutic options. *Blood Purif* 2002;20:252-61.
2. Shakil AO, Kramer D, Mazariegos GV, Fung JJ, Rakela J. Acute liver failure: clinical features, outcome analysis, and applicability of prognostic criteria. *Liver Transpl* 2000;6:163-9.
3. Stockmann HB, IJzermans JN. Prospects for the temporary treatment of acute liver failure. *Eur J Gastroenterol Hepatol* 2002;14:195-203.
4. Strain AJ, Neuberger JM. A bioartificial liver--state of the art. *Science* 2002;295:1005-9.
6. Sussman NL, Chong MG, Koussayer T, He DE, Shang TA, Whisennand HH, Kelly JH. Reversal of fulminant hepatic failure using an extracorporeal liver assist device. *Hepatology* 1992;16:60-5.
6. Kono Y, Yang S, Letarte M, Roberts EA. Establishment of a human hepatocyte line derived from primary culture in a collagen gel sandwich culture system. *Exp Cell Res* 1995;221:478-85.
7. McCloskey P, Edwards RJ, Tootle R, Selden C, Roberts E, Hodgson HJ. Resistance of three immortalized human hepatocyte cell lines to acetaminophen and N-acetyl-p-benzoquinoneimine toxicity. *J Hepatol* 1999;31:841-51.
8. McCloskey P, Tootle R, Selden C, Larsen F, Roberts E, Hodgson HJ. Modulation of hepatocyte function in an immortalized human hepatocyte cell line following exposure to liver-failure plasma. *Artif Organs* 2002;26: 340-8.
9. Rozga J, Williams F, Ro MS, Neuzil DF, Giorgio TD, Backfisch G, Moscioni AD, Hakim R, Demetriou AA. Development of a bioartificial liver: properties and function of a hollow-fiber module inoculated with liver cells. *Hepatology* 1993;17:258-65.
10. Flendrig LM, la Soe JW, Jorning GG, Steenbeek A, Karlsen OT, Bovee WM, Ladiges NC, te Velde AA, Chamuleau RA. In vitro evaluation of a novel bioreactor based on an integral oxygenator and a spirally wound nonwoven polyester matrix for hepatocyte culture as small aggregates. *J Hepatol* 1997;26:1379-92.
11. Wu L, Sun J, Wang L, Wang C, Woodman K, Koutalistras N, Horvat M, Sheil AG. Cryopreservation of primary porcine hepatocytes for use in bioartificial liver support systems. *Transplant Proc* 2000;32:2271-2.
18. Yagi T, Hardin JA, Valenzuela YM, Miyoshi H, Gores GJ, Nyberg SL. Caspase inhibition reduces apoptotic death of cryopreserved porcine hepatocytes. *Hepatology* 2001;33:1432-40.
12. Nyberg SL, Hibbs JR, Hardin JA, Germer JJ, Platt JL, Paya CV, Wiesner RH. Influence of human fulminant hepatic failure sera on endogenous retroviral expression in pig hepatocytes. *Liver Transpl* 2000;6:76-84.
13. Patience C, Takeuchi Y, Weiss RA. Infection of human cells by an endogenous retrovirus of pigs. *Nat Med* 1997;3:282-6.
14. Martin U, Kiessig V, Blusch JH, Haverich A, von der Helm K, Herden T, Steinhoff G. Expression of pig endogenous retrovirus by primary porcine endothelial cells and infection of human cells. *Lancet* 1998;352:692-4.
15. Wilson CA, Wong S, Muller J, Davidson CE, Rose TM, Burd P. Type C retrovirus released from porcine primary peripheral blood mononuclear cells infects human cells. *J Virol* 1998;72:3082-7.
16. Nyberg SL, Hibbs JR, Hardin JA, Germer JJ, Persing DH. Transfer of porcine endogenous retrovirus across hollow fiber membranes: significance to a bioartificial liver. *Transplantation* 1999;67:1251-5.
17. Martin U, Winkler ME, Id M, Radeke H, Arseniev L, Takeuchi Y, Simon AR, Patience C, Haverich A, Steinhoff G. Productive infection of primary human endothelial cells by pig endogenous retrovirus (PERV). *Xenotransplantation* 2000;7:138-42.
18. Heneine W, Tibell A, Switzer WM, Sandstrom P, Rosales GV, Mathews A, Korsgren O, Chapman LE, Folks TM, Groth CG. No evidence of infection with porcine endogenous retrovirus in recipients of porcine islet-cell xenografts. *Lancet* 1998;352:695-9.
19. Paradis K, Langford G, Long Z, Heneine W, Sandstrom P, Switzer WM, Chapman LE, Lockey C, Onions D, Otto E. Search for cross-species transmission of porcine endogenous retrovirus in patients treated with living pig tissue. The XEN 111 Study Group. *Science* 1999;285:1236-41.
20. Patience C, Patton GS, Takeuchi Y, Weiss RA, McClure MO, Rydberg L, Breimer ME. No evidence of pig DNA or retroviral infection in patients with short-term extracorporeal connection to pig kidneys. *Lancet* 1998; 352:699-701.
21. Samuel D, Ichai P, Feray C, Saliba F, Azoulay D, Arulnaden JL, Debat P, Gigou M, Adam R, Bismuth A, Castaing D, Bismuth H. Neurological improvement during bioartificial liver sessions in patients with acute liver failure awaiting transplantation. *Transplantation* 2002;73:257-64.
22. Demetriou AA, Brown RS, Busittil RW, Fair J, McGuire BM, Rosenthal P, et al. Prospective, randomized, multicenter, controlled trial of a bioartificial liver in treating acute liver failure. *Ann Surg* 2004;239:660-7
23. Ellis AJ, Hughes RD, Wendon JA, Dunne J, Langley PG, Kelly JH, Gislason GT, Sussman NL, Williams R. Pilot-controlled trial of the extracorporeal liver assist device in acute liver failure. *Hepatology* 1996;24:1446-51.
24. Millis JM, Kramer DJ, O'Grady J, Heffron T, Caldwell S, Hart ME, Maguire P. Results of phase I trial of the extracorporeal liver assist device for patients with fulminant hepatic failure. *Am J Transplantation* 2001;1 (suppl 1):391.
25. Sen S, Williams R, Jalan R. The pathophysiological basis of acute-on-chronic liver failure. *Liver* 2002;22 Suppl 2:5-13.
26. Arroyo V. Review article: albumin in the treatment of liver diseases-new features of a classical treatment. *Aliment Pharmacol Ther* 2002;16 Suppl 5:1-5.
27. Ruiz-del-Arbol L, Monescillo A, Jimenez W, Garcia-Plaza A, Arroyo V, Rodes J. Paracentesis-induced circulatory dysfunction: mechanism and effect on hepatic hemodynamics in cirrhosis. *Gastroenterology* 1997;113: 579-86.
28. Gines A, Fernandez-Esparrach G, Monescillo A, Vila C, Domenech E, Abecasis R, Angeli P, Ruiz-Del-Arbol L, Planas R, Sola R, Gines P, Terg R, Inglada L, Vaque P, Salerno F, Vargas V, Clemente G, Quer JC, Jimenez W, Arroyo V, Rodes J. Randomized trial comparing albumin, dextran 70, and polygeline in cirrhotic patients with ascites treated by paracentesis. *Gastroenterology* 1996;111:1002-10.
29. Sort P, Navasa M, Arroyo V, Aldeguer X, Planas R, Ruiz-del-Arbol L, Castells L, Vargas V, Soriano G, Guevara M, Gines P, Rodes J. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. *N Engl J Med* 1999;341: 403-9.
30. Duvoux C, Zanditenas D, Hezode C, Chauvat A, Monin JL, Roudot-Thoraval F, Mallat A, Dhumeaux D. Effects of noradrenalin and albumin in patients with type I hepatorenal syndrome: a pilot study. *Hepatology* 2002;36:374-80.
31. Ortega R, Gines P, Uriz J, Cardenas A, Calahorra B, De Las Heras D, Guevara M, Bataller R, Jimenez W, Arroyo V, Rodes J. Terlipressin therapy with and without albumin for patients with hepatorenal syndrome: results of a prospective, nonrandomized study. *Hepatology* 2002;36:941-8.
32. Curry S, Mandelkow H, Brick P, Franks N. Crystal structure of human serum albumin complexed with fatty acid reveals an asymmetric distribution of binding sites. *Nat Struct Biol* 1998;5:827-35.
33. Evans TW. Review article: albumin as a drug-biological effects of albumin unrelated to oncotic pressure. *Aliment Pharmacol Ther* 2002;16Suppl5: 6-11.
34. Awad SS, Rich PB, Kolla S, Younger JG, Reickert CA, Downing VP, Bartlett RH. Characteristics of an albumin dialysate hemodiafiltration system for the clearance of unconjugated bilirubin. *Asaio J* 1997;43: M745-9.
35. Awad SS, Sawada S, Soldes OS, Rich PB, Klein R, Alarcon WH, Wang SC, Bartlett RH. Can the clearance of tumor necrosis factor alpha and interleukin 6 be enhanced using an albumin dialysate hemodiafiltration system? *Asaio J* 1999;45:47-9.
36. Awad SS, Swaniker F, Magee J, Punch J, Bartlett RH. Results of a phase I trial evaluating a liver support device utilizing albumin dialysis. *Surgery* 2001;130:354-62.

37. Jalan R, Sen S, Steiner C, Kapoor D, Alisa A, Williams R. Extracorporeal liver support with molecular adsorbents recirculating system in patients with severe acute alcoholic hepatitis. *J Hepatol* 2003;38:24-31.
38. Stange J, Mitzner S. A carrier-mediated transport of toxins in a hybrid membrane. Safety barrier between a patients blood and a bioartificial liver. *Int J Artif Organs* 1996;19:677-91.
39. Stange J, Ramlow W, Mitzner S, Schmidt R, Klinkmann H. Dialysis against a recycled albumin solution enables the removal of albumin-bound toxins. *Artif Organs* 1993;17:809-13.
40. Stange J, Mitzner S, Ramlow W, Gliesche T, Hickstein H, Schmidt R. A new procedure for the removal of protein bound drugs and toxins. *Asaio J* 1993;39:M621-5.
41. Sen S, Ytrebo LM, Rose C, Fuskevaag OM, Davies NA, Nedredal GI, Williams R, Revhaug A, Jalan R. Albumin dialysis: a new therapeutic strategy for intoxication from protein-bound drugs. *Intensive Care Med* 2004.
42. Mitzner SR, Klammt S, Peszynski P, Hickstein H, Korten G, Stange J, Schmidt R. Improvement of multiple organ functions in hepatorenal syndrome during albumin dialysis with the molecular adsorbent recirculating system. *Ther Apher* 2001;5:417-22.
43. Sorkine P, Ben Abraham R, Szold O, Biderman P, Kidron A, Merchav H, Brill S, Oren R. Role of the molecular adsorbent recycling system (MARS) in the treatment of patients with acute exacerbation of chronic liver failure. *Crit Care Med* 2001;29:1332-6.
44. Stange J, Mitzner SR, Risler T, Erley CM, Lauchart W, Goehl H, Klammt S, Peszynski P, Freytag J, Hickstein H, Lohr M, Liebe S, Schareck W, Hopt UT, Schmidt R. Molecular adsorbent recycling system (MARS): clinical results of a new membrane-based blood purification system for bioartificial liver support. *Artif Organs* 1999;23:319-30.
45. Stange J, Mitzner SR, Klammt S, Freytag J, Peszynski P, Loock J, Hickstein H, Korten G, Schmidt R, Hentschel J, Schulz M, Lohr M, Liebe S, Schareck W, Hopt UT. Liver support by extracorporeal blood purification: a clinical observation. *Liver Transpl* 2000;6:603-13.
46. Mitzner SR, Stange J, Klammt S, Risler T, Erley CM, Bader BD, Berger ED, Lauchart W, Peszynski P, Freytag J, Hickstein H, Loock J, Lohr JM, Liebe S, Emmrich J, Korten G, Schmidt R. Improvement of hepatorenal syndrome with extracorporeal albumin dialysis MARS: results of a prospective, randomized, controlled clinical trial. *Liver Transpl* 2000;6: 277-86.
47. Heemann U, Treichel U, Loock J, Philipp T, Gerken G, Malago M, Klammt S, Loehr M, Liebe S, Mitzner S, Schmidt R, Stange J. Albumin dialysis in cirrhosis with superimposed acute liver injury: a prospective, controlled study. *Hepatology* 2002;36:949-58.
48. Novelli G, Rossi M, Pretagostini R, Poli L, Novelli L, Berloco P, Ferretti G, Iappelli M, Cortesini R. MARS (Molecular Adsorbent Recirculating System): experience in 34 cases of acute liver failure. *Liver* 2002;22 Suppl 2:43-7.
49. Novelli G, Rossi M, Pretagostini R, Novelli L, Poli L, Ferretti G, Iappelli M, Berloco P, Cortesini R. A 3-year experience with Molecular Adsorbent Recirculating System (MARS): our results on 63 patients with hepatic failure and color Doppler US evaluation of cerebral perfusion. *Liver Int* 2003;23 Suppl 3:10-5.
50. Isoniemi H. Current indications for albumin dialysis: Acute hepatic failure., In 4th International Symposium on Albumin Dialysis in Liver Disease, Rostock-Warnemunde, September 2002.
51. Felldin M, Friman S, Backman L, Siewert-Delle A, Henriksson BA, Larsson B, Olausson M. Treatment with the molecular adsorbent recirculating system in patients with acute liver failure. *Transplant Proc* 2003;35:822-3.
52. Felldin M, Friman S, Olausson M, Backman L, Castedal M, Larsson B, Henriksson BA, Siewert-Delle A. [Liver dialysis using MARS in acute hepatic failure. Promising results in a pilot setting]. *Lakartidningen* 2003; 100:3836-8, 3841.
53. Schmidt LE, Wang LP, Hansen BA, Larsen FS. Systemic hemodynamic effects of treatment with the molecular adsorbents recirculating system in patients with hyperacute liver failure: A prospective controlled trial. *Liver Transpl* 2003;9:290-7.
54. Falkenhagen D, Strobl W, Vogt G, Schrefl A, Linsberger I, Gerner FJ, Schoenhofen M. Fractionated plasma separation and adsorption system: a novel system for blood purification to remove albumin bound substances. *Artif Organs* 1999;23:81-6.
55. Rifai K, Ernst T, Kretschmer U, Bahr MJ, Schneider A, Hafer C, Haller H, Manns MP, Fliser D. Prometheus--a new extracorporeal system for the treatment of liver failure. *J Hepatol* 2003;39:984-90.
56. Krisper P, Stauber R, Haditsch B, Trauner M, Holzer H, D. Schneditz D. Mars versus prometheus: Comparison of reduction ratios (RR) as a measure of treatment dose in two different liver detoxification devices (abstract). *J Hepatol* 2004;40 (Suppl 1):69-70.
57. Sauer IM, Goetz M, Steffen I, Walter G, Kehr DC, Schwartlander R, Hwang YJ, Pascher A, Gerlach JC, Neuhaus P. In vitro comparison of the molecular adsorbent recirculation system (MARS) and single-pass albumin dialysis (SPAD). *Hepatology* 2004;39:1408-14.