

Extracorporeal Detoxification Using the Molecular Adsorbent Recirculating System for Critically Ill Patients with Liver Failure

STEFFEN R. MITZNER,* JAN STANGE,* SEBASTIAN KLAMMT,[†]
 PIOTR PESZYNSKI,* REINHARDT SCHMIDT,* and
 GABRIELE NÖLDGE-SCHOMBURG[‡]

*Divisions of *Nephrology and [†]Gastroenterology, Department of Medicine, and [‡]Department of Anesthesiology and Intensive Care Medicine, University of Rostock, Rostock, Germany.*

Abstract. Liver failure resulting from different causes and its concomitant complications represent difficult-to-treat conditions with high mortality rates, despite improved therapeutic modalities in intensive care medicine. The accumulation of albumin-bound metabolites that are normally cleared by the liver, such as bilirubin and bile acids, contributes substantially to the development of multiorgan dysfunction in these clinical situations. The molecular adsorbent recirculating system (MARS) represents a cell-free, extracorporeal, liver assistance method for the selective removal of albumin-bound substances. Moreover, it enables the removal of excess water and water-soluble substances via an inbuilt dialysis step. Since 1993, >400 patients have been treated in 53 centers in Europe, the United States, and Asia. Diseases treated with MARS included acute exacerbation of chronic hepatic failure, hepatorenal syndrome, acute hepatic failure, and primary nonfunction/poor

function after liver transplantation and major liver resection. Treatments were well tolerated. No severe adverse events were observed. Six- to 8-h MARS treatments resulted in significant ($P < 0.05$) removal of bilirubin, bile acids, tryptophan, short- and middle-chain fatty acids, aromatic amino acids, and ammonia. Clearance rates for strongly albumin-bound substances were between 10 and 60 ml/min. The removal of albumin-bound toxins resulted in decreases in hepatic encephalopathy, increases in mean arterial pressure, and improvements in kidney and liver function. In the first randomized clinical trial of the MARS method for treatment of the hepatorenal syndrome, significant prolongation of survival was observed for the MARS-treated group. It is concluded that the MARS method can contribute to the treatment of critically ill patients with liver failure and different underlying diseases.

Mortality rates for acute hepatic failure (AHF) and acute severe decompensation of chronic hepatic failure (AoCHF) resulting from different causes remain high, despite improved therapeutic modalities in modern intensive care medicine (1,2). Liver transplantation (Ltx) has greatly improved survival rates. For elective Ltx, 1-yr survival rates are up to 90% (3). However, mortality rates are much higher in AHF, ranging from 10 to 40% with Ltx and 50 to 90% without Ltx (4–6). Patients with AoCHF are normally not eligible for high-urgency Ltx. These patients frequently develop multiorgan failure, placing them at risk for systemic infections, cerebral edema, hemodynamic instability, coagulopathy, and various renal and metabolic complications (7).

The pathophysiologic features of the development of hepatic failure are not fully understood. Although the precipitating event is often well characterized (bacterial or viral infection, bleeding, or intoxication), the mechanisms leading to multiorgan

dysfunction/failure have not been completely elucidated. Internal intoxication with metabolites that are normally cleared from the circulation by the healthy liver seems to be one of the single most important mechanisms leading to hepatic encephalopathy (8–11), kidney failure (12–16), hyperdynamic circulation with systemic hypotension (17–20), liver insufficiency (detoxification, synthesis, and regulation) (21–25), and eventually death. Therefore, increased serum bilirubin levels are associated with increased mortality rates in AoCHF, as well as in AHF (1,26). Indeed, a number of metabolites accumulate in hepatic failure and are found in increased concentrations in these conditions (Table 1). Almost all of these substances are strongly protein bound. The most important transport protein for liver-bound metabolites is human serum albumin (HSA). Albumin has highly specialized binding sites for these substances (27). Among the substances that bind to albumin are most of the so-called protein-bound drugs, such as benzodiazepines (28). The pathophysiologic hypothesis that formed the basis for the molecular adsorbent recirculating system (MARS) method is the assumption that the accumulation of albumin-bound substances attributable to insufficient clearance by the failing liver results in elevated tissue levels of the subsequently toxic substances. Selective removal of the aforementioned substances from the blood should lead to redistribution and de-

Correspondence to Dr. Steffen R. Mitzner, Division of Nephrology, Department of Medicine, University of Rostock, E. Heydemann Strasse 6, 18055 Rostock, Germany. Phone and Fax: 49-381-494-7354; E-mail: steffen.mitzner@med.uni-rostock.de

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Table 1. Hydrophobic metabolites that use HSA as a transporter in extracellular fluid and plasma, with increased plasma concentrations in hepatic failure^a

Aromatic amino acids
Bile acids
Bilirubin
Copper (Wilson's disease)
Digoxin-like substances
Endogenous benzodiazepines
Indols
Mercaptans
Middle- and short-chain fatty acids
Nitric oxide
Phenols
Prostacyclins
Tryptophan

^a HSA, human serum albumin.

creases in the plasma and tissue concentrations of the metabolites. In the scope of this hypothesis, the ligands of the HSA molecule are referred to as albumin-bound toxins (ABT). Because ABT such as bilirubin and bile acids have hepatotoxic potential, *i.e.*, induction of hepatocyte apoptosis and necrosis (see above), it seemed reasonable to expect stabilization of liver function with time with sufficiently efficient and prolonged MARS treatments. The same is true for the kidney [in hepatorenal syndrome (HRS)]. It could be shown that the removal of bile acids and bilirubin decreased the degree of tubular necrosis (29).

Removal rates for single substances during MARS treatments and clinical effects observed in patients with hepatic failure resulting from different causes are presented and discussed in this review.

Materials and Methods

MARS Treatment

MARS is a liver support system developed to support excretory liver function. It consists of elements from extracorporeal renal replacement techniques such as hemodialysis and ultrafiltration, as well as adsorption. It contains no biologic components, such as hepatocytes. The method uses an albumin-enriched dialysate to facilitate the removal of ABT. It contains three different fluid compartments ("circuits"), *i.e.*, the blood circuit, an albumin circuit, and an open-loop, single-pass dialysate circuit. MARS requires a standard dialysis machine or a continuous venovenous hemofiltration (CVVH) device (*e.g.*, BM 25; Edwards Life Sciences GmbH, Munich, Germany) to control the blood and dialysate circuits. An extra device (MARS monitor; Teraklin AG, Rostock, Germany) is necessary to control and monitor the closed-loop albumin circuit. The latter circuit connects the blood and dialysate circuits.

The blood circuit uses a venovenous access (double-lumen catheter) and is driven by the blood roller pump of the dialysis machine and the CVVH monitor. The blood flow rate is 150 to 250 ml/min, depending on the hemodynamic status of the patient. Blood is passed through a non-albumin-permeable high-flux dialysis membrane (MARSFlux; Teraklin) (Figures 1 and 2). The albumin circuit, con-

taining 20% HSA, is driven by a roller pump of the MARS monitor at 150 ml/min. The dialysate HSA is passed through the dialysate compartment of the blood dialyzer (MARSFlux) and subsequently regenerated by dialysis against a bicarbonate-buffered dialysate (dialysate circuit), followed by passage through two sequential columns; the first contains uncoated charcoal, and the second contains an anion exchanger resin (30). Heparin is used as an anticoagulant. The activated clotting time is maintained between 160 and 190 s throughout the treatment.

Review of Clinical Trials

Clinical trials and pilot studies from different centers using the technique of albumin dialysis were reviewed.

MARS was normally performed intermittently for 6 to 8 h/d. However, continuous treatment for up to 24 h/d was performed in individual cases. Treatment pauses of 24 h were allowed for hemodialysis or hemofiltration sessions or other diagnostic or therapeutic interventions. The number of single treatment days ranged between 1 and 24 d, with an average of 5 to 6 d/patient.

In Vivo Removal Capacities of Hemodiafiltration and MARS

To compare the efficacy of MARS with that of hemodiafiltration with respect to single-substance clearance, we determined the pre- and post-treatment concentrations of different albumin-bound substances (bilirubin, bile acids, fatty acids, tryptophan, and aromatic amino acids). No branched-chain amino acids were administered during the assessment period. The same type of dialysis membrane was used throughout the experiments. Flow rates, ultrafiltration rates, and dialysate flow rates were kept at the aforementioned levels. The Fischer ratio (branched-chain/aromatic amino acid ratio) was calculated for evaluation of the course of the amino acids.

Statistical Analyses

All results were expressed as means \pm SD. Because the data were normally distributed, the *t* test was used to analyze differences between mean values for each variable before and after treatment (paired

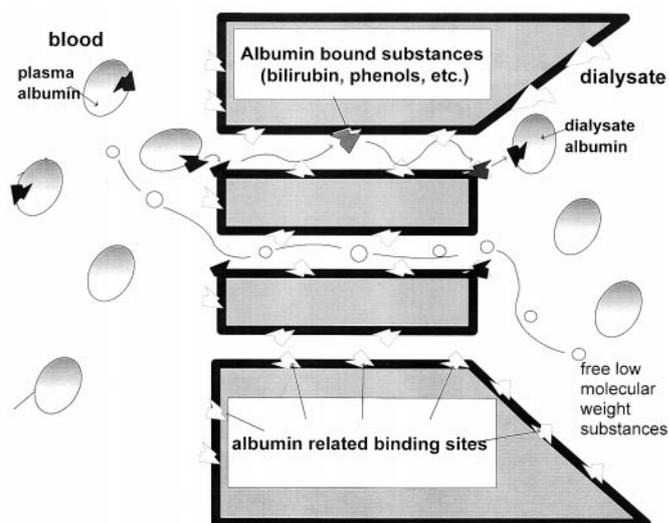


Figure 1. Schematic drawing of the transport of albumin-bound and water-soluble substances through the MARSFlux membrane.

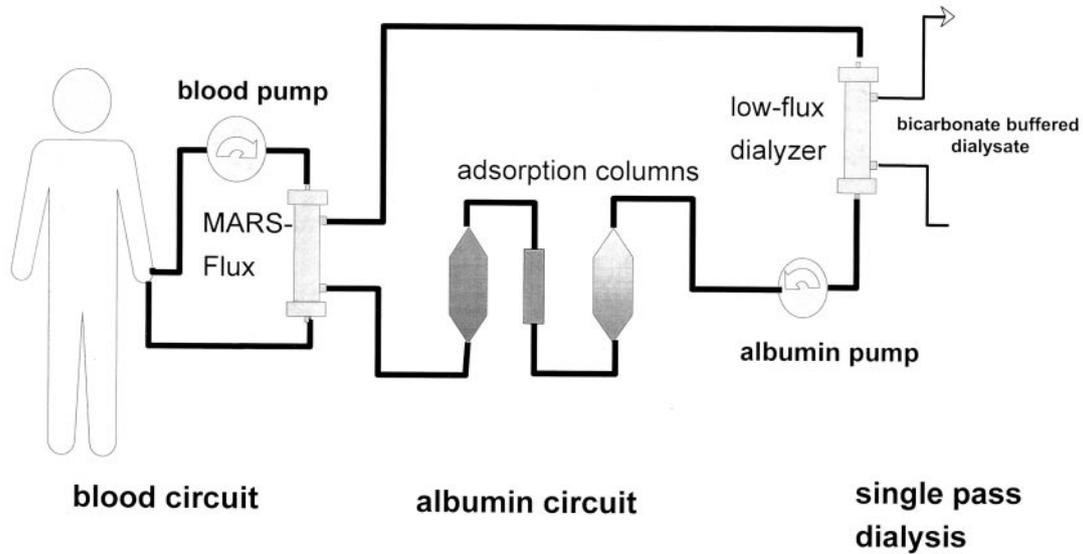


Figure 2. Schematic drawing of the molecular adsorbent recirculating system (MARS).

t test) and between the groups (unpaired *t* test). The 5% probability level was regarded as statistically significant.

Results

Substance Clearance

Significant removal of water-soluble and albumin-bound substances was observed during MARS therapy, by our group and by other groups. An overview of single substances measured and found to be significantly removed during treatment using the albumin dialysis concept is presented in Table 2. Clearance rates for ABT ranged between 10 and 60 ml/min.

Clinical Comparison of the Removal Efficacy of Hemodiafiltration versus MARS

Under comparable conditions (flow rates, membrane, and treatment times), significantly higher removal rates for bilirubin

Table 2. Substances with significant decreases in plasma concentrations during albumin dialysis treatments

Substance	References
Water-soluble	
ammonia	31-33
creatinine	34-36,39
urea	33
Albumin-bound	
bilirubin	34-39
bile acids	35, this report
tryptophan	40
middle- and short-chain fatty acids	This report
aromatic amino acids (increase in Fischer index)	31,40
mediators (tumor necrosis factor- α , interleukin-6)	41
copper	37
diazepam	42

($P < 0.001$), bile acids ($P < 0.001$), middle- and short-chain fatty acids ($P < 0.05$), and tryptophan ($P < 0.05$) were found for MARS, compared with hemodiafiltration. The difference in Fischer ratios missed the level of significance ($P = 0.05$). Figures 3 to 7 provide the results obtained from the pretreatment/post-treatment comparison of blood concentrations of different albumin-bound substances.

Clinical Effects

Summary of Effects. Various clinical effects were observed primarily for patients with AoCHF. However, the first reports of data for patients with AHF and primary nonfunction also demonstrated favorable clinical effects. These included improvements in mental status, liver detoxification and synthesis functions, hemodynamic status (increase in mean arterial pressure), and kidney function (Table 3). Additional effects not listed in Table 3 included the marked regression of pruritus in patients with AoCHF (Huster D, Berr F, unpublished observa-

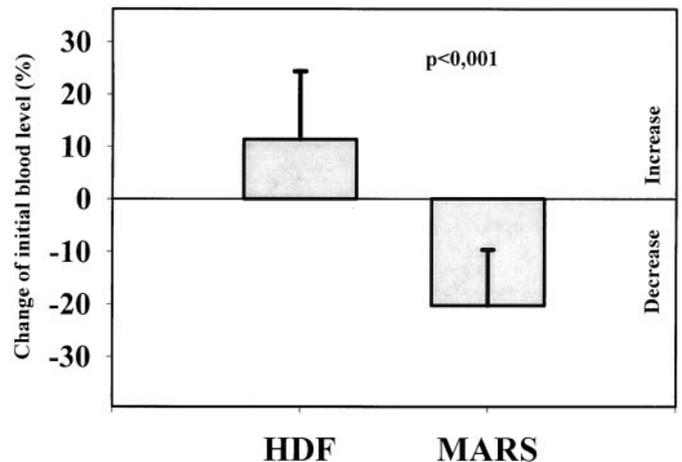


Figure 3. Percent change in the plasma concentration of bilirubin after a 6-h treatment with hemodiafiltration (HDF) or MARS.

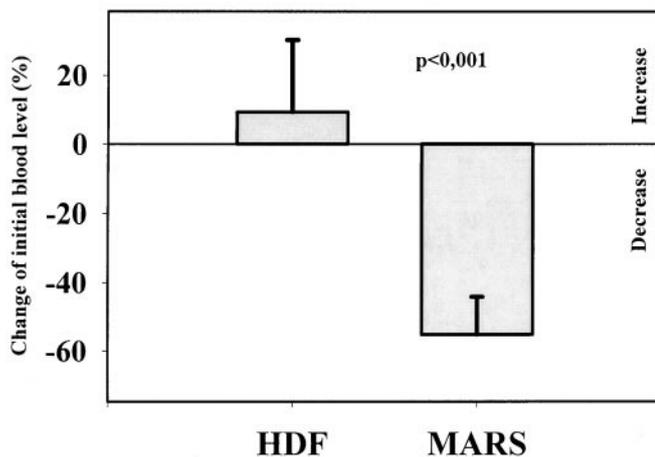


Figure 4. Percent change in the plasma concentration of bile acids after a 6-h treatment with HDF or MARS.

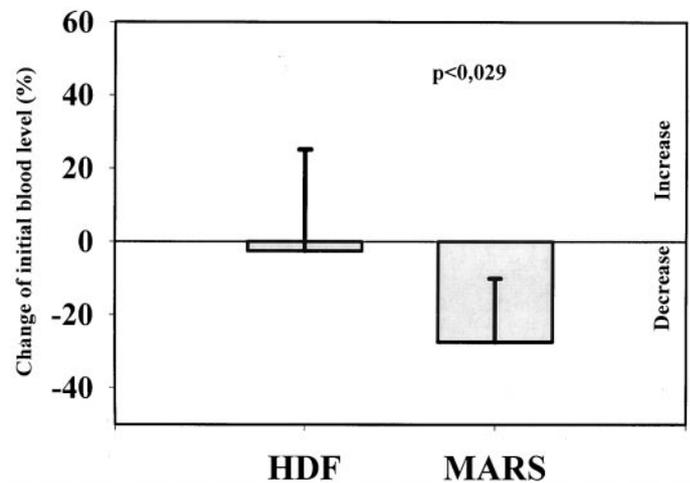


Figure 6. Percent change in the plasma concentration of tryptophan after a 6-h treatment with HDF or MARS.

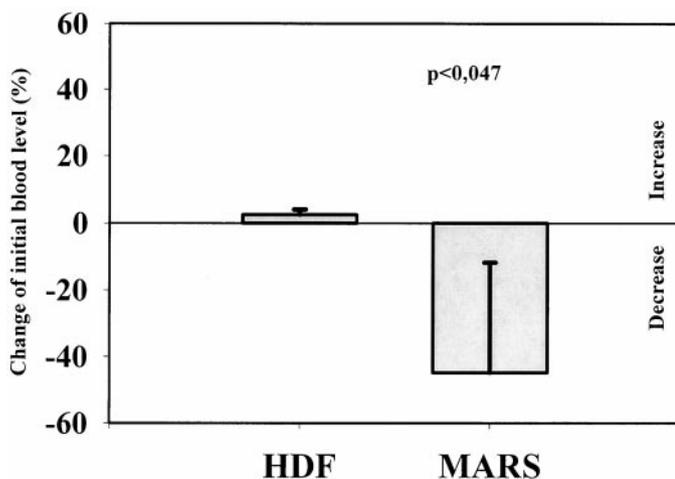


Figure 5. Percent change in the plasma concentration of short- and middle-chain fatty acids after a 6-h treatment with HDF or MARS.

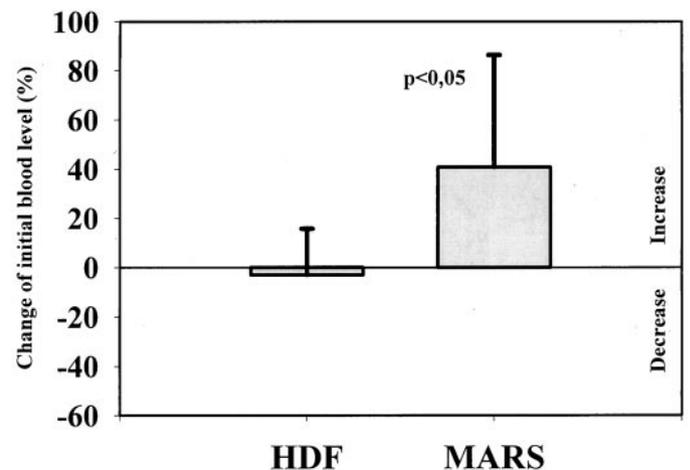


Figure 7. Percent change in the Fischer index (branched-chain/aromatic amino acid ratio) after a 6-h treatment with HDF or MARS.

tions) (39) and increases in serum sodium levels during a series of MARS treatments for patients with HRS (34).

Cerebral Function. The degree of hepatic encephalopathy was decreased in patients with AHF, as well as in patients with AoCHF (31,35,37,39).

Hemodynamic Function. The mean arterial pressure was increased in patients with AoCHF (34,36). This was probably attributable to increases in systemic vascular resistance (36).

Kidney Function. MARS treatment improved kidney function for patients with AoCHF and HRS (34,36,39).

Liver Function. Protein synthesis improved during the treatment phase, and plasma antithrombin III levels, prothrombin activity, and Factor VII levels were increased. Cholinesterase levels were also increased (31-35,39).

Child-Turcotte-Pugh (CTP) scores decreased because of improved liver synthesis, decreased encephalopathy grades, and decrease in degree of ascites. The significant decrease in bilirubin concentrations did not contribute to changes in CTP scores, because plasma concentrations after the MARS treat-

ments were still elevated (>3 mg/dl = 3 points). Interestingly, the CTP scores remained low and even exhibited additional decreases after the end of the MARS treatments (35).

In cell culture experiments performed with primary rat hepatocytes, the cytotoxicity of plasma from patients with AoCHF was markedly decreased (increases in viability and cytochrome P450 activity) after the patients were treated with MARS (35).

Adverse Events

Almost all groups reported smooth safe performance of the MARS technique (31,32,34,35,37-39). No adverse events attributable to MARS treatments were documented.

Discussion

Different approaches to supportive liver therapy have been used in the past decade, including extracorporeal whole-liver perfusion (43), hepatocyte transplantation (44), and extracorporeal artificial and bioartificial devices (45-48). Potential problems arising from the use of bioartificial liver systems

Table 3. Clinical effects observed during a series of albumin dialysis treatments for patients with AHF, AoCHF, or PNF after Ltx^a

Organ Effect	AHF	AoCHF	PNF
Brain function	Decrease in HE grade (31,37)	Decrease in HE grade (31,35,39)	
Liver synthesis, ascites	Factor VII increase (31), INR decrease (32)	Factor VII increase (31), ATIII increase (35), prothrombin activity (quick) increase (34,35,39), cholinesterase increase (33,39), ascites regradient (35)	INR increase (32)
Kidney function		Urine volume increase for four of eight patients with HRS (34), plasma renin decrease, urine volume increase (36)	
Cardiovascular function		SVR increase, MAP increase (36), MAP increase (34)	
Survival	HepB, successful bridging to Ltx (32)	Significant improvement in HRS (34), two of five patients with HRS recovered (36)	1 case: recovery after 24 MARS treatments (32); 3 cases: split-liver recipients (38)

^a HE, hepatic encephalopathy; HRS, hepatorenal syndrome; MARS, molecular adsorbent recirculating system; SVR, systemic vascular resistance; INR, international normalized ratio; ATIII, antithrombin III; HepB, hepatitis B; Ltx, liver transplantation; AHF, acute hepatic failure; AoCHF, acute on chronic hepatic failure; PNF, primary nonfunction; MAP, mean arterial pressure.

include antibody formation and complement activation after repeated treatments with bioartificial liver systems using xenogenic cells (49), viral transfer to patients (50), and induction of proinflammatory cytokines such as tumor necrosis factor- α and interleukin-6 (51).

Results obtained to date with the albumin dialysis MARS indicate that the hypothesis made in the beginning holds true. The removal of ABT seems to contribute to improvement in organ function and survival rates in different forms of liver failure.

However, no comprehensive pathophysiologic explanation can now be provided for the clinical and biochemical effects observed during the course of MARS treatment. Current knowledge regarding the removal kinetics of albumin-bound substances and some clinical effects of the MARS method give rise to different hypotheses that attempt to elucidate the mechanism of action of the MARS method in HRS. Possible mechanisms include the following.

Removal of Nitric Oxide

Nitric oxide (NO) is thought to be one of the factors responsible for the vasodilation and hyperdynamic status observed in hepatic failure and HRS. Plasma NO can act as a potent vasodilator (17,18,20). It is transported primarily bound to serum albumin as an *S*-nitrosothiol (19). Therefore, a possible mechanism of action of the MARS method could involve the removal of plasma NO. The increase in systemic vascular resistance and mean arterial pressure observed in the MARS-treated patients with AoCHF and HRS (34,36) might be explained by such a mechanism.

Increase in Binding Capacity for Toxic Metabolites

ABT were found to compete with tryptophan for free binding capacity at the receptor sites of the albumin molecule in uremic patients (52). The MARS concept of albumin dialysis was shown to facilitate the *in vivo* removal of strongly albumin-bound uremic compounds such as 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (53). Therefore, the increase in free binding capacity at the surface of the albumin molecule by MARS treatment could decrease the concentration of free toxic metabolites via binding to serum albumin. This again may lead to decreased tissue concentrations of these substances, facilitating organ recovery. The intravenous infusion of HSA was shown to have beneficial effects on the course and outcome of spontaneous bacterial peritonitis (54) and resulted in improved response rates and prevention of recurrence of ascites (55). This again may indicate that the increase in the total binding capacity of the serum albumin pool of the patient can have beneficial effects for patients with ascites and hepatorenal failure.

Removal of Bilirubin, Bile Salts, and Bile Acids

Bilirubin, bile salts, and bile acids have toxic potential and can impair liver and kidney function. Bile salts were shown to cause lactate dehydrogenase leakage from human endothelial cells *in vitro*. Prostacyclin release from the cells was decreased (56). Moreover, bile salts can cause hepatocyte death by inducing mitochondrial permeability transition (25) and apoptosis in rodent hepatocytes (22). In the kidney, bile acids disturb renal water and electrolyte handling by blocking the Na⁺/H⁺-antiporter in the tubule and thus impairing intracellular pH

regulation. Bile acids induce damage to tubular cell membranes by stimulating oxygen radical generation (12,13). High bilirubin levels are a predictor of death in acute renal failure, because of acute tubular necrosis (14). Although serum concentrations of up to 15 mg/dl did not induce changes in renal function in patients with obstructive jaundice, higher concentrations in patients with hypoalbuminemia were associated with decreased urinary sodium excretion, free water clearance, negative water clearance, renal blood flow, and creatinine clearance (15). Bilirubin can also cause tubular damage. Kamisako *et al.* (16) demonstrated a positive effect of (enzyme-triggered) bilirubin removal on hepatic and renal function in a rat model of obstructive jaundice. Those authors concluded that the systemic reduction of serum bilirubin concentrations might contribute to normalization of the urinary levels of prostaglandins and thromboxane B₂, to decreases in serum bile acid levels, and to improvement of the hepatic energy charge in obstructive jaundice. Moreover, they concluded that preoperative improvement of jaundice might be beneficial for patients with obstructive jaundice (16).

The removal of bilirubin and bile acids/salts during MARS treatment could therefore decrease the toxic effects that higher concentrations of these compounds exert on liver and kidney function and could thus contribute to improvements in organ function, as observed by different groups (31-36,39).

Removal of Aldosterone and Other Vasoactive Hormones

The renin-angiotensin-aldosterone system, the sympathetic nervous system, and arginine vasopressin are responsible for sodium and water retention in patients with cirrhosis (57). Except for plasma renin analysis (36), no hormone determinations were performed in the trials reviewed. However, we started to analyze plasma aldosterone concentrations and plasma renin activity in patients treated with MARS. Preliminary data obtained in a single case of a patient with type I HRS who was treated with MARS indicated that single-pass reductions of plasma aldosterone levels of 40% and plasma renin activity of 11% in the extracorporeal circulation could be achieved. These reductions may lead to substantial decreases in the systemic concentrations and activities of these hormones with treatment times of 6 to 8 h. For eight patients with AoCHF who were treated once with MARS for 10 h, significant reductions in plasma renin concentrations were observed (36). These data provide an initial indication of possible effects of MARS treatments on plasma concentrations of the aforementioned hormones, which may in part explain the clinical improvements observed (*e.g.*, in HRS).

The parallel removal of water-soluble and albumin-bound substances seems to be one of the major advantages of the MARS approach, because both groups can substantially contribute to the clinical situation in advanced AHF/AoCHF. Probably the most important water-soluble substance is ammonia, because it plays a pivotal role in the development of cerebral edema and hepatic encephalopathy (58). Approximately 55% of all patients referred to specialized centers with AHF develop some type of renal failure (hepatorenal failure or

drug-induced renal failure) (59). In cases that need renal replacement therapy it might be reasonable to use the albumin dialysis MARS as a first choice for extracorporeal treatment rather than hemodialysis or CVVH, as (1) MARS allows the removal of ABT in addition to the clearance of water-soluble factors and ultrafiltration, and (2) the risk/benefit ratio of the procedure is in favor of the MARS method.

In summary, we conclude that MARS treatment can substantially contribute to the treatment of patients with liver failure. However, more multicenter, randomized, clinical trials, with well defined patient groups and standardized outcome measures, will be essential for proper evaluation of the clinical value of this system and other artificial or bioartificial devices.

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