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Hemodynamic Parameters in a Surgical Devascularization Model of Fulminant Hepatic Failure in the Minipig

E. KIESLICHOVÁ, M. RYSKA[1], T. PANTOFLÍČEK[1], O. RYSKA[1], R. ZAZULA, J. SKIBOVÁ

Department of Anesthesiology, Resuscitation and Intensive Care and [1]Transplant Surgery Department, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

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Summary

Animal models of fulminant hepatic failure (FHF) are important for studying the pathophysiology of this process and for evaluation of the efficacy of artificial and bioartificial liver support systems. In experiments, hemodynamic parameters were monitored in a group of minipigs with FHF induced by surgical devascularization, and compared with those in a control group. During the experiment, animals were analgosedated and were on mechanical lung ventilation. Crystalloid and colloidal solutions were administered and norepinephrine in continuous infusion was applied if mean arterial pressure (MAP) decreased below 60 mm Hg despite adequate intravascular volumes. An increase in heart rate, and decreases in MAP and systemic vascular resistance, compared with the baseline, occurred in the FHF group from 6 h after surgery. A comparison of FHF and control groups revealed no significant differences in systemic vascular resistance and MAP until after 12 h after surgery (systemic vascular resistance index: 953 FHF vs. 1658 controls; $p<0.05$; MAP: 58.1 FHF vs. 76 controls; $p<0.05$). No significant differences in CI were seen between the FHF group and controls. FHF animals survived for about 13 h after surgery, i.e. a period, which we consider long enough to test a support device. The parameters are believed to be quite adequate, as we were able to maintain satisfactory hemodynamic stability in all experimental animals with induced acute hepatic failure.

Key words

Fulminant/acute hepatic failure • Devascularization surgical pig model • Hemodynamic parameters

Introduction

Fulminant hepatic failure (FHF) is a rarely occurring devastating disease associated with high mortality. It is characterized by rapid onset of severe hepatic dysfunction in an individual without previous liver disease, development of jaundice, encephalopathy, and coagulopathy (Bernuau *et al.* 1986). Loss of hepatic function results in rapid development of multiorgan failure (Munoz 1993). Despite advances in support therapy, FHF is still associated with mortality as high as 80 % (Gill *et al.* 2001), unless emergency liver transplantation is performed in the presence of a grim prognosis (Lee 1994, Sechser *et al.* 2001). Development of FHF is accompanied by significant hemodynamic instability (Mas and Rodes 1997). Considerable

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

splanchnic and systemic vasodilation is a typical feature of FHF combined with hyperdynamic circulation and a low arteriovenous difference in oxygen content. Tissue hypoxia, which may develop despite seemingly adequate cardiac output and arterial oxygenation, contributes to the development of multiorgan failure (Bilhari *et al.* 1985, 1991). The pathogenesis of microcirculatory impairment is not clear, with a role possibly played by vasoactive substances from necrotic liver, cytokines produced by macrophages in response to systemic endotoxemia, and endothelial damage by free oxygen radicals (Kaptanoglu and Blei 2000). Another factor with an effect on circulation may be the occlusion of microcirculation by microthrombi (Ellis and Wendon 1996) formed as a result of activation and consumption of platelets and increased endothelial adhesion of leukocytes (Bilhari *et al.* 1986).

As an immediate availability of a suitable organ is an exception rather than the rule, liver transplantation is performed in approximately 10-20 % of these patients (Kamohara *et al.*1998, Stockmann *et al*. 2000). Hence the current interest in temporary liver support designed to bridge the patient until liver transplantation and/or, ideally, to provide the time necessary for spontaneous or stimulated regeneration of the native liver (Abrahamse *et al.* 2002).

A condition for experimental testing of support devices is the existence of a simple and reproducible model of FHF (Kaptanoglu and Blei 2000). Extensive research has been conducted to develop suitable and reproducible animal model of FHF. The animal models for the treatment of acute liver failure (ALF) include anhepatic and devascularization procedures (de Groot *et al*. 1987) and hepatotoxic drug administration. Administration of various drugs has been the most common method for inducing FHF in animals (Kelly *et al.* 1992). Induction of FHF with drugs is less specific and reproducible than in the devascularization model, because other organs also were affected (Miller *et al*. 1976). We used irreversible devascularization model of ALF in the minipig (Ryska *et al.* 2004*)*. The aim of our experiment was to maintain satisfactory hemodynamic stability of an animal in a surgical devascularization model of FHF and to compare hemodynamic parameters in a group of FHF animals and a control group.

Methods

Materials

The study includes 20 adult minipigs with body weight of 25-30 kg. In 15 minipigs, FHF was induced by surgical devascularization involving ligation of the hepatic artery and the portal vein combined with an end-to-side portocaval anastomosis. In 5 of these minipigs, intracranial pressure was measured continually using a cranial intraparenchymal optic fiber probe (Codman, Johnson and Johnson, USA). A control group included five animals undergoing only laparotomy. The onset of FHF in the minipig was documented by hypoglycemia, liver test elevation, a decrease of prothrombin time and coagulation factor activities and development of intracranial hypertension. Figure 1 shows the development of intracranial pressure (ICP) in the group with FHF and ICP monitoring. The increase of intracranial pressure in the group with FHF is linear, significant change, compared to the normal value was found after 3 hours after surgery. On postmortem examination liver appeared necrotic as a whole.

All experiments were carried out in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC).

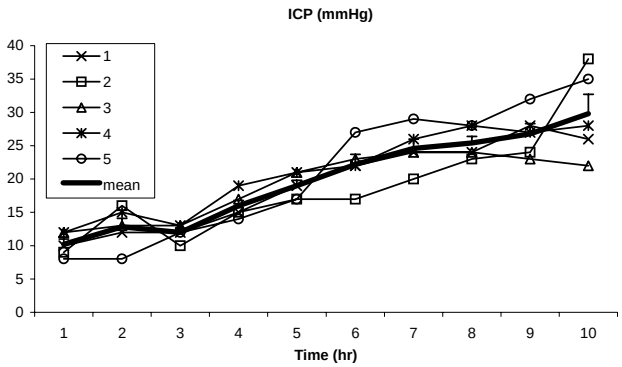

Fig. 1. Individual and mean values of intracranial pressure (ICP in mmHg) of minipigs in the group with FHF measured after surgery.

Anesthesia

Prior to surgery, the animal fasted for 12 h with fluid intake *ad libitum*. On the day of surgery, the animal was weighed and measured. Twenty minutes prior to surgery, the animals were premedicated with ketamine 10 mg/kg, atropine 0.1 mg/kg and azaperone 5 mg/kg intramuscularly. After placing the minipig on the operating table, a cannula was inserted into an ear vein and anesthesia was induced with ketamine 5 mg/kg and metomidate 2-5 mg/kg. A probe was placed on the ear for pulse oximetry (SpO_2). Following endotracheal intubation, the animal was connected to a ventilator

(Servoventilator, Siemens-Elema, Sweden), relaxed with pipecuronium at a dose of 50 μg/kg and mechanically ventilated with a mixture of oxygen and nitrous oxide in 1:1 ratio. Anesthesia was complemented with repeated doses of fentanyl (100 μg i.v.) at 30-min intervals. Heparin at a dose of 500 IU was administered to prevent thromboembolism and famotidine to prevent stress-related ulceration. Antibiotic-based prophylaxis was provided with amoxicilin 1.2 g. After induction of general anesthesia, an arterial catheter (18 G, Braun, Germany) was inserted into a femoral artery for direct blood pressure measurement. A double-lumen catheter (7 Fr, Arrow, USA) was inserted into a femoral vein for drug and infusion administration. A flow-directed thermodilution catether (7 Fr, Arrow, USA) was inserted into the pulmonary artery *via* the right internal jugular vein to measure hemodynamic parameters. ECG, mean arterial pressure (MAP), central venous pressure (CVP), mean pulmonary artery pressure (MPAP), end-tidal CO_2, central and peripheral body temperature, and pulse oximetry were monitored continually throughout the procedure using a monitoring system (Marguette, USA). During the procedure, warm crystalloid solutions 1-2 ml/kg/h and, if needed, colloids 3 ml/kg were administered to compensate for the loss of fluids and blood.

Postoperative management

After the surgery, animals were placed on their sides and warmed. General anesthesia was continued with continuous analgosedation, with all animals further intubated and ventilated with a mixture of oxygen and air, with a 0.5 fraction of oxygen. Analgosedation was performed using drugs with predominantly extrahepatic route of elimination. We used a combination of propofol at an initial dose of 6 mg/kg/h, remifentanil at an initial dose of 1 μg/kg/min, and medetomidine at an initial dose of 3 μg/kg/h. Central venous pressure (CVP) was maintained above 3-5 mm Hg and pulmonary artery wedge pressure (PCWP) above 5 mm Hg by crystalloids and/or colloid administration. Whenever MAP decreased below 60 mm Hg, norepinephrine was started in continuous infusion at an initial dose of 0.05 μg/kg/min. In the presence of glycemia below 3.5 mmol/l caused by liver failure onset (Bernstein and Tripodi 1998) 40 % glucose was administered as continuous infusion in an effort to maintain glycemia within a range of 3.3-4.9 mmol/l (Seidel 1972). No blood or blood products were administered during the whole experiment.

After the experiment and sacrificing the animals by a lethal dose of thiopenthal followed by potassium chloride, autopsy was performed. The correct position of all catheters was checked, and the organs were visually inspected.

Hemodynamic monitoring

Hemodynamic parameters were measured and recorded upon induction of general anesthesia (T1), at the end of the procedure (T2) and, subsequently, at 3-hour intervals during the experiment (T3, T6, T9, T12). Heart rate (HR), MAP, CVP and MPAP were measured continuously, with pulmonary capillary wedge pressure (PCWP) determined at regular 3-hour intervals and cardiac output (CO) by the thermodilution technique. The resulting CO was determined as a mean of three consecutive values. Cardiac index (CI) and the systemic vascular resistance index (SVRI) were calculated (Table 1). We compared changes in hemodynamic parameters during this time in FHF and control animals, and the FHF group with the control group.

Table 1. Hemodynamic calculations (Scharf 1992)

BSA	(body surface area) m^2: weight (kg) $^{0.425}$ + height (cm)$^{0.725}$ + 7.184×10^{-3}
MAP	(mean arterial pressure) mm Hg: (systolic + 2 × diastolic)/3
MPAP	(mean pulmonary artery pressure) mm Hg: (systolic + 2 × diastolic)/3
SVRI	(systemic vascular resistance index) dyne.sec.cm^5/m^2 = (MAP – CVP)/CI × 80
CI	(cardiac index) l/min/m^2 = CO/BSA

Statistical analysis

Parameters are presented as means ± SE. Data were analyzed using the Mann-Whitney rank test and Wilcoxon's sign rank test. As the number of animals in either group was smaller than 20, non-parametric tests were used for statistical analysis. For each analysis, $p<0.05$ was considered statistically significant.

Results

Animals with FHF survived for about 13 hours (range 11.7 to 15.2 h). Control animals were sacrificed after a corresponding period. No significant changes were demonstrated in all parameters monitored in the control

animals over the same time; support with norepinephrine was not required to maintain MAP. In the group of minipigs with FHF, norepinephrine in continuous infusion was required starting at 4 h after surgery in order to maintain MAP over 60 mm Hg. At 6 h postoperatively, there was a decrease in MAP compared with baseline in the FHF group: 84.7 vs. 101.7 ($p<0.05$), and MAP continued to decline after further 3 h: 67.7 vs. 84.7 ($p<0.05$). At 12 h postoperatively, MAP was substantially lower than at baseline: 58.1 vs. 101.7 ($p<0.05$). When comparing the FHF and control groups, the difference was not significant until 12 h postoperatively: 58.1 vs. 76 ($p<0.05$) (Fig.2). Heart rate in the FHF group rose at 6 h after surgery ($p<0.05$). It was significantly higher compared with baseline at 9 and 12 h ($p<0.01$). Significant differences between the groups were seen at 6, 9, and 12 h ($p<0.01$) after the end of the surgery (Fig. 3). A decrease in CVP over time in FHF was observed at 6 hours postoperatively ($p<0.001$), with no further significant decline thereafter. A difference between FHF and controls was not seen until 12 h postoperatively ($p<0.05$). No significant alterations were noted in FHF over the time. Differences between the groups tended to be significant at 6, 9, and 12 h ($0.10<p<0.05$). Systemic vascular resistance index decreased in FHF at 6, 9, and 12 h ($p<0.01$) compared with the baseline; the decrease was not significant between 6 and 9 hours. The difference between the groups was not evident until 12 h after surgery ($p<0.05$) (Fig. 4). MPAP showed no changes over time. When comparing the groups, MPAP was higher in FHF at 3 and 6 h ($p<0.05$), and at 9 and 12 h postoperatively ($p<0.01$). Cardiac index (CI) in FHF did not change over the time. No significant CI differences were seen when comparing the groups (Fig. 5).

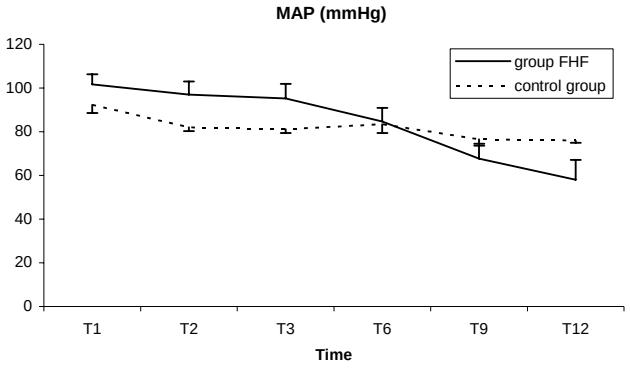

Fig. 2. Mean arterial pressure (MAP) in FHF and control animals.

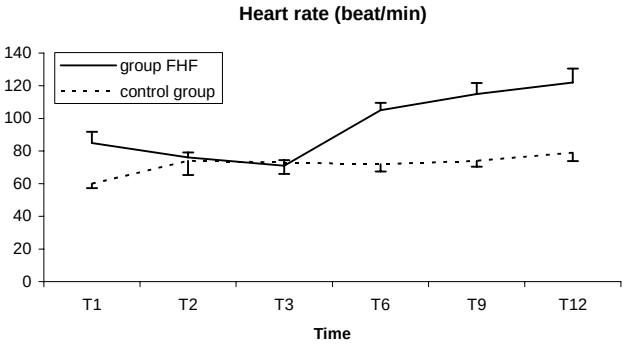

Fig. 3. Heart rate in the group minipigs with FHF and in control animals

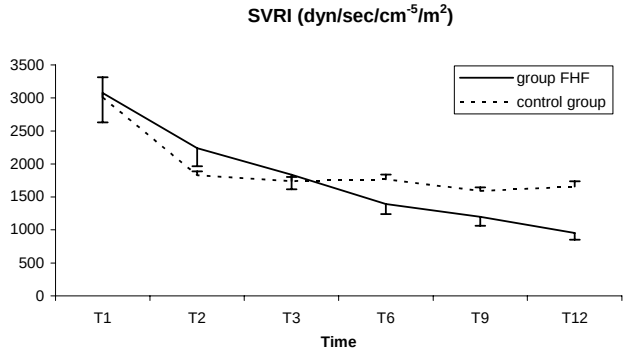

Fig. 4. Systemic vascular resistance index (SVRI) in FHF and control animals.

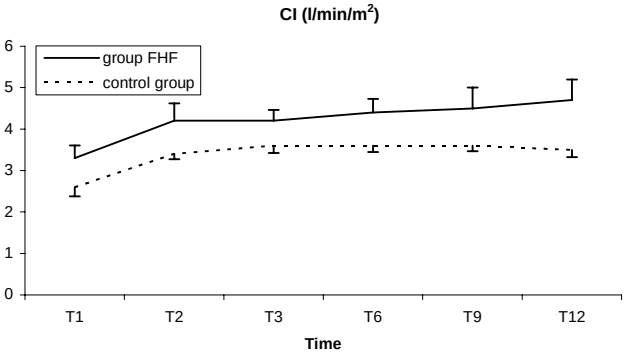

Fig. 5. Cardiac index (CI) in FHF and control animals.

Discussion

Hemodynamic parameters of animals with FHF were compared with those of the control undergoing laparotomy only. All measurements were performed in sedated animals. Analgosedation eliminates the factor of stress and the effect of stimulation when taking care of animals (Hess *et al*. 1984). On the other hand, an effect of anesthesia and postoperative analgosedation on hemodynamic parameters should be expected (Hanning 1996). To achieve higher hemodynamic stability,

analgosedation included medetomidine, a highly specific α_2-adrenergic receptor agonist, showing marked antiarrhythmic action (Hayashi *et al*. 1991). A typical feature of FHF are atrial and ventricular arrhythmias usually associated with electrolyte dysbalance and acid base imbalance (Bernstein and Tripodi 1998). During the experiment, a tendency toward supraventricular tachycardia developed at 6 h after surgery, but no ventricular arrhythmia was noted. Concomitant administration of medetomidine also enabled to reduce the doses of analgesics and sedatives (Maze and Tranquilli 1991). Throughout the experiment, animals were connected to mechanical lung ventilation without any signs of acute lung injury (Neuhaus and Blumhardt 1993).

Liver function deterioration in FHF is associated with progressive vasodilation and hypotension (Bernstein and Tripodi 1998). Hypotension remains one of the most frequent management problems in FHF, and full hemodynamic monitoring is essential. Initial management is directed towards optimizing the volume status. If hypotension persists in the face of optimal volume loading and cardiac output, vasopressors are required (Shakil *et al*. 1999, Riordan and Williams 2000). In our experiment, animals with FHF received norepinephrine in continuous infusion in all cases where there was a decrease in MAP below 60 mm Hg occurred despite adequate intravascular filling. Norepinephrine infusion was started at an initial dose of 0.05 μg/kg/min at about 4 h postoperatively. The dose was continuously adjusted according to MAP where every effort was made to maintain MAP for cerebral perfusion pressure to be over 50 mm Hg (Wendon *et al*. 1994, Blei 1998, Larsen *et al.* 2000). No therapeutic interventions other than administration of crystalloid and colloidal solutions, norepinephrine in continuous infusion and hypertonic glucose were used. It should be pointed out that no blood was administered.

In conclusion, using a surgical devascularization model of fulminant hepatic failure, satisfactory hemodynamic stability could be maintained in minipigs in experiment. This clinically relevant model allows the evaluation of new biotechnology, which may be found useful in the management of FHF in man.

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Reprint requests

E. Kieslichová, Department of Anesthesiology, Resuscitation and Intensive Care, Institute for Clinical and Experimental Medicine, Vídeňská 1958/9, 140 21 Prague 4, Czech Republic. E-mail: evki@medicon.cz