ORIGINAL ARTICLE

Fractionated plasmatic separation and adsorption does not alter haemodynamic parameters in experimental acute liver failure

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Abstract

OBJECTIVE: Acute liver failure (ALF) is a rare disease with a bad prognosis. Its start is accompanied by haemodynamic instability. The aim of our study was to evaluate the influence of fractionated plasmatic separation and adsorption (FPSA) on body haemodynamics using a large animal experimental model of ALF.
METHODS: ALF was induced by the devascularisation of 21 laboratory pigs. FPSA was applied in 14 animals and seven animals formed a control group. Values of systemic vascular resistance index (SVRI), heart rate (HR), pulmonary artery wedge pressure (PAWP) and cardiac index (CI) at hours 3, 6, 9 and 12 of the experiment were compared. The values from laboratory tests conducted with FPSA-treated vs. untreated ALF animals were compared using Student's *t*-test, paired or unpaired, as required, and Mann-Whitney *U*-test using EXCEL and QUATRO spreadsheet applications.
RESULTS: We found no significant differences in mean arterial pressure, SVRI, or plasma lactate ($p>0.05$) in the FPSA-treated group but there was a significant decrease($p<0.05$) in intracranial pressure (ICP). Furthermore, we observed a significant decrease in HR at hour 3. A significant increase in CI at hour 9 and a significant decrease in pulmonary artery wedge pressure at hours 6 and 12 were also observed.
CONCLUSION: Our study of FPSA application (Prometheus device) for treatment of experimental ALF in a large animal model did not confirm the earlier reported development of changes in body haemodynamics.

Abbreviations:

ALF	- acute liver failure
FPSA	- fractionated plasmatic separation and adsorption
HR	- heart rate
SVRI	- systemic vascular resistance index
CI	- cardiac index
MAP	- mean arterial pressure
ICP	- intracranial pressure
PAWP	- pulmonary artery wedge pressure
PCA	- portocaval anastomosis
PCV	- pressure-controlled ventilation
TV	- tidal volume
PEEP	- positive end-expiratory pressure
$paCO_2$	- arterial partial pressure CO_2
CVP	- central venous pressure
TT	- body temperature
$ETCO_2$	- end-tidal carbon dioxide concentration in the expired air
SpO_2	- peripheral oxygen saturation
MOSR	- multiorgan dysfunction syndrome

INTRODUCTION

Acute liver failure (ALF) is a relatively rare disease with a high mortality rate. In addition to encephalopathy and frequent fatal infectious complications, this syndrome is often accompanied by circulatory instability. The pathophysiology of hyperkinetic circulation accompanied by a microcirculation injury appearing in ALF is not yet fully explained. An increased load of substances with a vasodilatatory potential including nitric oxide and the release of other vasoactive substances (cytokines) in the context of an immunological response to systemic endotoxaemia (Kaptanoglu & Blei 2000), together with the increased sensitivity of injured veins to vasoconstrictor agents (Wiest & Groszmann 2002) play a significant role in the development of the clinical signs of ALF.

Fractionated plasmatic separation and adsorption (FPSA) therapy using a Prometheus device (Fressenius, Germany) is a new method used in the management of acute liver failure (Laleman *et al.* 2006; Rifai & Manns 2006). It eliminates both water-soluble and albumin-bound toxic substances (Leckie *et al.* 2012; Rademacher *et al.* 2012). The influence of FPSA on haemodynamics in the course of ALF has not yet been satisfactorily examined.

The aim of our study was to evaluate the influence of FPSA on haemodynamic parameters in a large animal model of ALF induced using the method of devascularisation (Ryska *et al.* 2004).

MATERIAL AND METHODS

I. The experimental surgical model of ALF

Altogether, 21 laboratory pigs (body weight 25 to 40 kg) with ALF induced by the devascularisation method consisting in hepatic artery ligation and end-to-side portacaval anastomosis (PCA) (Ryska *et al.* 2004) were used in the study. The onset of ALF was documented by the appearance of hypoglycaemia (<3.5 mmol/l). FPSA therapy was then applied in 14 pigs, with the remaining animals used as a control group. The experiment was terminated 12 hours after the onset of ALF.

II. Anaesthesia

Animals were premedicated with an intramuscular (IM) injection of a mixture of 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 dexmedetomidine (Domitor, Pfizer, USA), which was given to the animals 20 minutes before the surgical operation.

General anaesthesia was induced with an intravenous (IV) injection of a mixture of 4 μg/kg fentanyl (Fentanyl Torrex, Torrex, Chiesi, Austria) and 0.3 mg/kg etomidate (Hypnomidate, Janssen Pharmaceutica, Belgium); animals were then intubated. After intubation, artificial pulmonary ventilation was started using a Siemens Servo 900 C ventilator (Siemens, Elema, Sweden) using pressure-controlled ventilation (PCV), with Fi 0_2 0.4, PEEP 4, F: 16/minute; 6 to 8 ml/kg tidal volume (TV) and normocapnia were maintained ($PaCO_2$ 4.6–5.3 kPa). For general anaesthesia, isoflurane (Forane, Abbott Laboratories, UK) with air mixture inhalation and a continuous infusion of a combination of 6–10 μg/kg fentanyl (Fentanyl Torrex, Torrex Chiesi, Austria) and 1 μg/kg/minute dexmedetomidine (Domitor, Pfizer, USA) were applied. Animals were also given boluses of 0.02 mg/kg pipecuronium bromide (Arduan, Gedeon Richter, Hungary), a muscle relaxant, during the surgical operation. Amoxicillin (1.2 g) and Quamatel (1 amp.) were used to prevent infection and development of a stress ulcer. The femoral vein and the femoral artery were cannulated, to connect the animals to the Prometheus device and for blood pressure monitoring. The internal jugular vein was surgically exposed and a thermodilution pulmonary artery catheter (7F Arrow, USA) was inserted to monitor haemodynamic parameters.

III. Post-surgical care and monitoring

Each operated animal was placed on its left side and analgo-sedated using medicaments with an extrahepatic elimination pathway. An intracranial pressure (ICP) intraparenchymal sensor (Codman, Johnson-Johnson, USA) was inserted into the right cerebral hemisphere of animals to provide ICP monitoring. Mean arterial pressure (MAP) was maintained at a level higher than 60 mmHg by continuously infusing crystalloidal and colloidal solutions. When ineffective, we used continuous infusion of noradrenaline (Noradrenalin, Leciva, Czech Republic). Hypoglycaemia (less than 3.5 mmol/l glucose, the main clinical sign of ALF (Rahman & Hodgson 2001), was compensated by continuous infusion of 40% glucose solution to achieve normal blood glucose concentration (3.5–5.0 mmol/l).

Heart rate [HR], MAP, and central venous pressure [CVP]) were monitored continuously. The cardiac index (CI), systemic vascular resistance index (SVRI), and pulmonary artery wedge pressure (PAWP) were monitored at the beginning of the experiment and at 3-hour intervals (0, 3, 6, 9, and 12 hours). Body temperature (TT), end-tidal carbon dioxide concentration in the expired air ($ETCO_2$), and peripheral oxygen saturation (SpO_2) were measured throughout experiment. Blood was sampled to determine the acid-base balance, glycaemia, complete blood morphologic characteristics, haemocoagulation parameters, and plasma activities of liver enzymes and plasma concentrations of creatinine, urea, and ammonia.

IV. Connection to FPSA system

In the process of FPSA, the Prometheus device eliminates both water-soluble and albumin-bound toxic substances. The device consists of a FMC 4008H dialyser, a module for fractionated plasmatic separation using an albumin filter, and two adsorbers (Prometh1 and Prometh2). Only molecules smaller than 250 kD can pass through Prometh1. The adsorption of bile acids, aromatic amino acids, and phenolic substances is thus carried out. Prometh2, equipped with a 100 µm styrene-divinylbenzene copolymer space grid, captures negatively charged ligands (non-conjugated bilirubin) (4, 5).

The blood pump of the Prometheus device delivered the animals' right pulmonary artery blood to an AlbuFlow filter that separated blood plasma, together with the albumin fraction and albumin-bound substances. The blood was then passed along the secondary circuit into two adsorbers (Prometh1 and Prometh2). The albumin, cleared of the bound substances, was then returned to blood. Subsequently, the full blood was passed into a classical dialyser (HiFlux) that removed, by haemodialysis, the substances dissolved in water. The cleared blood was returned to the animals via the left femoral vein. During FPSA therapy we used heparin as an anticoagulant.

At the end of experiments, the animals were killed using a lethal dose of thiopental and potassium chloride solution. Necropsies were then conducted for visualisation of the abdominal viscera and control of the positioning of the catheters used.

V. Statistical processing of the data

The values from laboratory tests conducted with FPSA-treated vs. untreated ALF animals were compared using Student's *t*-test , paired or unpaired, as required, and Mann-Whitney *U*-test using EXCEL and QUATRO spreadsheet applications.

VI. 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 Animals Decree No. 207/2004 Coll.

The studies were performed in accordance with guidelines and practices established by the Animal Care and Use Committees of the Institute for Clinical and Experimental Medicine and of the 2nd Faculty of Medicine, Charles University, Prague.

RESULTS

A total of 25 laboratory pigs were used for experiments; ultimately, the data for 21 were analysed and statistically evaluated. Two animals were excluded from the experiment because of premature death due to unmanageable postoperative bleeding. Intracranial bleeding in places of sensor insertion precluded ICP monitoring in two other animals during the second stage of the experiment. All the other animals of the control and experimental groups stayed alive until hour 12 and were fully monitored. The mean duration of surgical operations was 1 h 40 min. FPSA therapy started on the average 3 h 17 min (range: 2 h 15 min to 4 h 20 min) after the completion of devascularisation and lasted for an average of 5 h 54 min (range: 5 h 45 min to 6 h). In order to keep MAP at a level higher than 60 mm Hg, small doses of noradrenaline (0.05 to 0.15 µg/kg/min) were administered to four pigs. Higher doses of noradrenaline were applied to all pigs of the control group throughout experiments. No significant difference in noradrenaline dosage needed was noted between the control and experimental group undergoing FPSA therapy.

During the whole experiment, we noted no significant differences in MAP, SVRI, or lactate ($p>0.05$) between the two groups. No significant difference in HR was noted between the experimental and control group, respectively, at hours 6 (78.3±20 vs. 84.7±9.5), 9 (87.4±22.2 vs. 85.7±11), and 12 (100.1±37.3 vs. 101.7±8.8). A significant decrease in HR values from the initial control was noted in the experimental group under FPSA therapy ($p<0.05$) at hour 3 (75.3±10.9 vs. 64.3±12.5). Compared with the FPSA therapy group, we noted significantly lower CI values in the control group at hour 9 (3.5±0.5 vs. 4.7±1.5). No significant difference in CI values was noted between the groups at hours 3 (3.9±1 vs. 3.6±0.7), 6 (4.2±1.1 vs. 3.6±0.6), and 12 (3.7±0.9 vs. 3.5±0.5). Compared with the FPSA therapy group, we noted significantly lower PAWP values in the control group at hours 6 (4.8±4.1 vs. 8.1±2.1) and 12 (4±2.9 vs. 7±2.9). Compared with the FPSA therapy group, no significant difference in PAWP values was noted in the control group at hours 3 (5.9±3.8 vs. 7.3±2.9) and 9 (6.4±4.1 vs. 9.3±4; see Figures 1–3). On the contrary, we found significantly lower ICP values in the FPSA group compared with the control group ($p<0.05$) between hours 9 and 12 (19.1±4.09 vs. 24.1±2.85 mmHg at hour 9, 21.9±3.63 vs. 25.1±2.19 mm Hg at hour 10, 22.5±3.98 vs. 26.3±3.5 mmHg at hour 11, and 24.0±4.66 vs. 29.8±5.88 mmHg at hour 12).

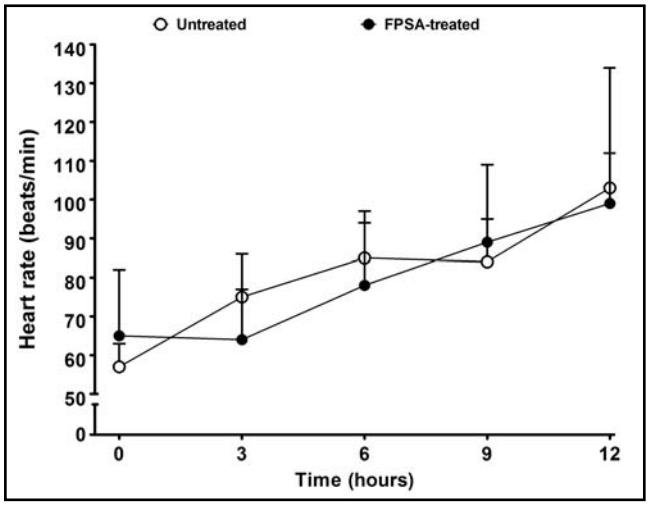

Fig. 1. Comparison of heart rate (HR) values in the group of animals with ALF and treated using a FPSA device, and in the control group with ALF and no therapy.

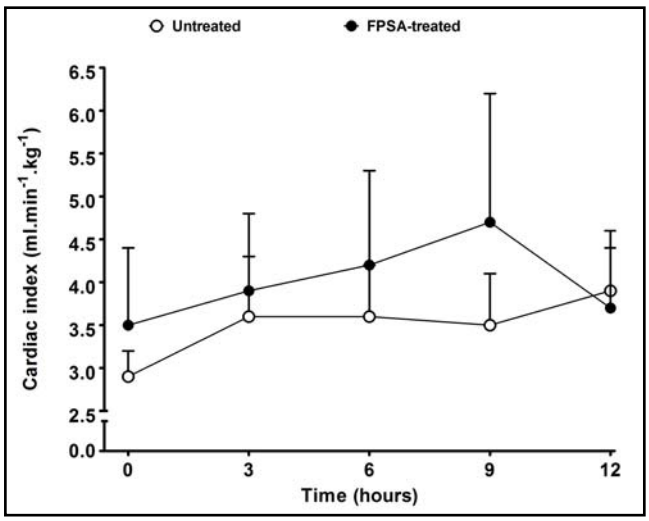

Fig. 2. Comparison of cardiac index (CI) values in the group of animals with ALF and treated using a FPSA device, and in the control group with ALF and no therapy.

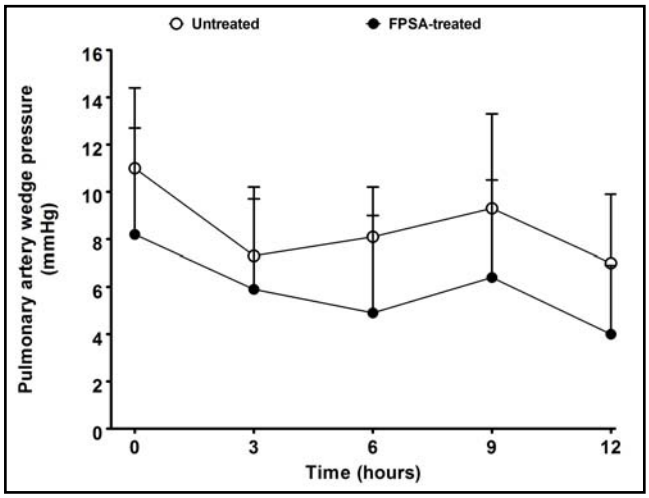

Fig. 3. Comparison of pulmonary artery wedge pressure (PAWP) values in the group of animals with ALF and treated using a FPSA device, and in the control group with ALF and no therapy.

DISCUSSION

I. The effect of acute liver failure on body haemodynamics

ALF is a relatively rare disease with a very bad prognosis. The onset of ALF is accompanied by rapid systemic haemodynamic dysfunction and instability, manifested through hyperkinetic circulation (Auzinger & Wendon 2008). Hyperkinetic circulation is characterised by splanchnic vasodilatation (Ytrebo *et al.* 2006). SVRI and MAP decrease contrasting with HR and CI increase. Organ and tissue hypoxia, depending on organ low oxygen uptake (associated with increasing CI), leads to splanchnic vasoconstriction, hypoperfusion of organs, and the development of multiple organ dysfunction syndrome (Auzinger & Wendon 2008). ALF has recently been proved to be accompanied by a hypermetabolic state in the hepatosplanchnic area, characterised by increased glycolysis and hyperlactataemia. This hypermetabolic state enhances organ hypoxia and hyperlactataemia in ALF (Clemmesen *et al.* 2004; Murphy *et al.* 2001).

II. Fractionated plasmatic separation and adsorption

The elimination of some albumin-bound vasoactive substances could influence the aforementioned pathophysiological processes (Laleman *et al.* 2006; Rahman & Hodgson 2001). FPSA execution was not accompanied by problems in our study. Appropriate perfusion parameters which provided satisfactory rapid circulation in the primary blood circuit prevented blood clotting; heparin-protamine was used as an anticoagulant. Never in our studies had an experiment to be interrupted or terminated because of blood clotting in the system. The fact that albumin and albumin-bound toxins are transported directly to the place of their adsorption is an advantage of FPSA systems. The adsorption process is an integral part of FPSA procedure and there is no need of further processing. On the contrary, the Molecular Adsorbent Recirculating System (MARS), a previous non-biological support system, does need such addition. The use of the FPSA method is, therefore, simpler and cheaper.

III. FPSA in experimentally induced ALF and its effect on haemodynamics

In the course of experiments a significant decrease in SVRI together with a significant increase in HR and CI were observed in both groups. This observation agrees with the pattern of haemodynamic changes in ALF described earlier (Kieslichova 2005). Some workers reported a significant increase in MAP during therapy with MARS (Schmidt *et al.* 2001; Steiner & Mitzner 2002). We observed no significant differences in haemodynamic parameters between our FPSA-treated and untreated group, in agreement with the data on FPSA treatment of ALF described by Dethloff *et al.* (2008).

It will be noticed that application of the FPSA method for treatment of experimental ALF in large laboratory animals was found to decrease ICP (Ryska *et al.* 2009). In the present study, in order to maintain the necessary MAP level, we continuously administered small doses of noradrenaline so that the MAP levels were not significantly different in FPSA-treated compared to the control group. The elimination, using the FPSA method, of vasoactive substances, responsible for the development of multiorgan dysfunction syndrome (MOD) (Bergis *et al.* 2012; Stadlbauer *et al.* 2006), possibly support the assumption of the influence of FPSA on haemodynamic parameters during treatment with the FPSA system. Other studies are necessary to verify this theory.

CONCLUSION

Our study of FPSA application (Prometheus device) for treatment of experimental ALF in a large animal model did not confirm the earlier reported development of changes in body haemodynamics.

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Conflict of Interest

The authors declare no conflict of interest.

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