International Journal of Cardiology 168 (2013) 4699–4704

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Cytokine removal on extracorporeal life support for treatment of acute endotoxemia: A randomized controlled animal study

Patrick W. Weerwind [a], Frederik H. van der Veen [a], Sandro Gelsomino [a,b,*], Naveen Gaddehosur Nagaraj [a], Orlando Parise [a], Roberto Lorusso [b], Gian Franco Gensini [b], Jos G. Maessen [a]

[a] Cardiovascular Research Institute Maastricht – CARIM, Maastricht University Medical Centre, The Netherlands
[b] Heart and Vessels Department, Careggi Hospital, Florence, Italy

ARTICLE INFO

Article history:
Received 25 January 2013
Received in revised form 18 June 2013
Accepted 20 July 2013
Available online 25 July 2013

Keywords:
Sepsis
Resin adsorption
Cytokine removal
Extracorporeal life support

ABSTRACT

Background: We prospectively evaluated the effectiveness of resin adsorption incorporated in an extracorporeal life support (ELS) circuit in an animal model of sepsis for removal of cytokines and prevention of hemodynamic deterioration during the treatment of septic shock.
Methods: Twelve female landrace pigs were randomly assigned to two groups, a study group($n = 6$), treated with high-flow resin adsorption (300 mL/min) and ELS, and a control group ($n = 6$), treated only with ELS. Septic shock was induced by intravenous 0.02 μg/kg/min infusion was of *Escherichia coli* lipopolysaccharide (LPS). Measurements were carried out in the study group at baseline, at the end of LPS injection(t_0) at 30(t_1), 60(t_2), 90(t_3) and 120 min (t_4) and 60 min after stopping resin-adsorption (t_5). In the control group measurements were performed at baseline (t_0), t_1 and only t_2, as no control animal survived beyond this latter experimental timepoint.
Results: The final population consisted of 9 animals, five in the study group and 4 in the control group. Plasma values of both tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) were reduced during resin-adsorption (t_1–t_4) while these mediators increased in controls undergoing ELS only. With a clearance of TNF-α of 15,233 pg/min and IL-6 of 10,233 pg/min, 79.2% of TNF-α and 95.3% of IL-6 produced were adsorbed. Systemic vascular resistance decreased significantly in both groups at t_0. While it further was reduced during the control experiments at t_1 and t_2, it returned to normal in the study animals. Cardiac output increased at t_0, t_1 and t_2 in the control experiments. In contrast, in study animals after a peak at t_0, it returned to the baseline value and did not vary thereafter.
Conclusions: Combined resin-adsorption and ELS improved hemodynamics resulting from effective removal of inflammatory mediators in a pig model of septic shock.

1. Introduction

Septic shock is still the leading cause of mortality and morbidity in intensive care units and the improvement of its prognosis remains a major therapeutic challenge [1–3]. Sepsis appears to develop as a result of the host response to infection [4] and circulating pro-inflammatory and anti-inflammatory mediators contribute to the complex cascade of events which leads to cell and organ dysfunction and, in many cases, to death [5,6]. Therefore, extracorporeal blood purification therapies have been proposed to improve outcomes for patients with sepsis.

Nonetheless, techniques such as high-volume hemofiltration, cascade hemofiltration, plasmapheresis, high-adsorption hemofiltration-high-cutoff and hemodialysis/hemofiltration have shown theoretical as well as practical limitations and they have not entered into mainstream clinical practice around the world [7].

High-volume plasma filtration in combination with sorbent adsorption (coupled plasma filtration absorption [CPFA]) was studied as a treatment option in severe sepsis to achieve higher mediator clearance and mass removal rates [8]. Nevertheless this technique, while achieving 100% single-pass removal of tumor necrosis factor (TNF) and interleukin-10 (IL-10), was not able to lower the circulating levels of these two cytokines [9], thus suggesting unsatisfactory clearance of excess mediators using relatively low flow rates (30–40 mL/min) in patients with sepsis [9].

Therefore, the aim of this study was to evaluate, in an animal model of sepsis, the effectiveness of high-flow resin adsorption (300 mL/min) associated with extracorporeal life support (ELS), in removing cytokines

* Corresponding author at: Dept. of Cardiothoracic Surgery, Cardiovascular Research Institute Maastricht – CARIM, Universiteitssingel 50, 6229 ER Maastricht, The Netherlands.
E-mail address: sandro.gelsomino@libero.it (S. Gelsomino).

0167-5273/$ – see front matter
http://dx.doi.org/10.1016/j.ijcard.2013.07.175

and preventing hemodynamic deterioration during the treatment of septic shock.

2. Methods

The Institutional Ethics Committee approved the study. Care and handling of the animals was in compliance with the Italian national guide for the care and use of laboratory animals (DL. 116/1992) and the recommendations of the European Economic Community (86/609/CEE) for the care and use of laboratory animals.

2.1. Subjects and experimental protocol

Twelve health-certified domestic landrace pigs (female, mean weight 78 ± 6 kg) were investigated. Animals were randomly assigned to two groups: a study group (n = 6), treated with resin-adsorption and ELS and a control group (n = 6), treated only with ELS.

All pigs underwent injection of lipopolysaccharide (LPS) from *Escherichia coli* (0111: B4, Sigma-Aldrich Co., St Louis, MO, USA). When the systolic pulmonary pressure reached 45 mmHg, the infusion was stopped so as to limit the increase of the right ventricular afterload [10].

The rate of LPS infusion was 0.02 μg/kg/min, the infusion time was 15.9 ± 2.2 min in the study group and 14.9 ± 2.3 in the control Group (p = 0.47). The total LPS dose received was comparable in the two groups (25.0 ± 5.3 μg vs. 23.5 ± 3.5 μg in the study pigs and controls, respectively, p = 0.57).

Animals were then connected to the ELS system and a pump flow of 2.5 L/min was initiated upon hemodynamic deterioration, and maintained for 3 h. The CPFA kit (Bellco S.r.l., Mirandola, MO, Italy) was installed in the study group (Fig. 1) and a separate roller pump assured a fixed flow of 300 mL/min through the adsorption filter. Plasma filtration was continued for 2 h, followed by one hour on ELS support only. The control animals were connected to the ELS system without incorporation of a CPFA kit.

Measurements were carried out in the study group at baseline, at the end of LPS injection (t_0) at 30 (t_1), 60 (t_2), 90 (t_3) and 120 min (t_4) from the end of LPS injection and on ELS and CPFA and 60 min after stopping filtration (t5). In the control group measurements were performed at baseline, t_0, t_1 and t_2 as no control animal survived beyond this experimental timepoint. At each experimental time three consecutive samples were taken to provide more reliable parameters assessments. All deaths of the control group occurred between T2 and T3, as said, for a rapid refractory cardiocirculatory deterioration leading to ELS failure due to marked reduction of the venous return likely secondary to severe septic vasoplegia and metabolic acidosis, both unresponsive to mechanical circulatory support.

2.2. Surgical procedure

Animals were premedicated with 4 mg/kg azaperone IM (Stressnil, Janssen Pharmaceutical, Beerse, Belgium) and 0.05 mg/kg atropine sulfate IM (Galenica Senese, Siena, Italy). Anesthesia was induced with intravenous ketamine (11 mg/kg, Parke Davis-Pfizer, Karlsruhe, Germany) and diazepam (0.25 mg/kg, Roche, Fontenay-sous Bois, France). Animals were intubated during spontaneous breathing with a 7.5 Fr orotracheal tube and placed on mechanical ventilation. Tidal volume, respiratory rate, and percent inspired oxygen were adjusted to maintain arterial pH between 7.38 and 7.42, partial carbon dioxide pressure between 35 and 40 torr (35–40 mmHg) and arterial oxygen saturation >98%. Paralysis was obtained with 0.1 mg/kg pancuromium bromide (Organon N.V., Oss, The Netherlands). Anesthesia was maintained with nitric oxide (50%) and sevorane (1–2%). Continuous intravenous propofol infusion (20 mL/hour, Braun, Melsungen, Germany) was started before opening the chest. A warming blanket maintained the animal body temperature at 37 ± 0.5C°.

After heparin administration (bolus 150 IU/kg) the activated clotting time (ACT) was measured (Hemochron, International Technidyne Corporation, Edison, NJ, USA) and kept between 200 and 300 seconds.

A median sternotomy was performed, and the pericardium was opened longitudinally. A 5-mm graft (Vascutek, Inchinnan, UK) was anastomized to the ascending aorta and a 18 Fr EOPA CAP arterial cannula (Medtronic Inc., Minneapolis, MN, USA) was connected to the aortic graft. A 19 Fr Bio Medicus multi-stage venous cannula (Medtronic Inc.) was inserted into the right femoral vein.

At termination of the experiments the surviving animals were euthanized with an intracardiac bolus of potassium chloride (30 mEq/10 mL, Bioindustria L.I.M, Novi Ligure, Italy).

2.3. Monitoring

The electrocardiogram (ECG) was continuously monitored in a standard DII lead and oxygen saturation was monitored a by pulse-oxymeter applied to the left ear (ML320/E, AD Instruments, Oxfordshire, UK). Rectal temperature was monitored continuously (ML312 T, AD Instruments). A 7.5 Fr Swan-Ganz catheter (Arrow International) was advanced into the pulmonary artery via the right jugular vein and a pressure transducer (SP 844, AD Instruments) was connected to the distal lumen.

Continuous monitoring of oxygen saturation, rectal temperature, arterial blood pressure, ECG, central venous pressure and pulmonary pressure during the procedure was via a 16-channel based acquisition system (Power Lab, AD Instruments).

The left femoral artery was cannulated with a standard 18-gauge catheter and connected to the Most-Care™ monitor, which was powered by the Pressure Recording Analytical Method (PRAM) [10] via a standard pressure monitoring set (Edwards Life Sciences, Irvine, CA, USA). The resulting signal was processed by Most-Care™ (Release 1.00A, Vytech Healthcare, Padova, Italy) for the determination of the cardiac output (CO). Most-Care™ was connected to a computer for continuous recording of the hemodynamic data.

Lactate and pH were measured routinely in samples presented for blood gas analyses (ABL800 Flex, Radiometer Medical ApS, Brønshøj, Denmark).

The systemic vascular resistances (SVR) and the pulmonary vascular resistance (PVR) were calculated from standard formulae [11].

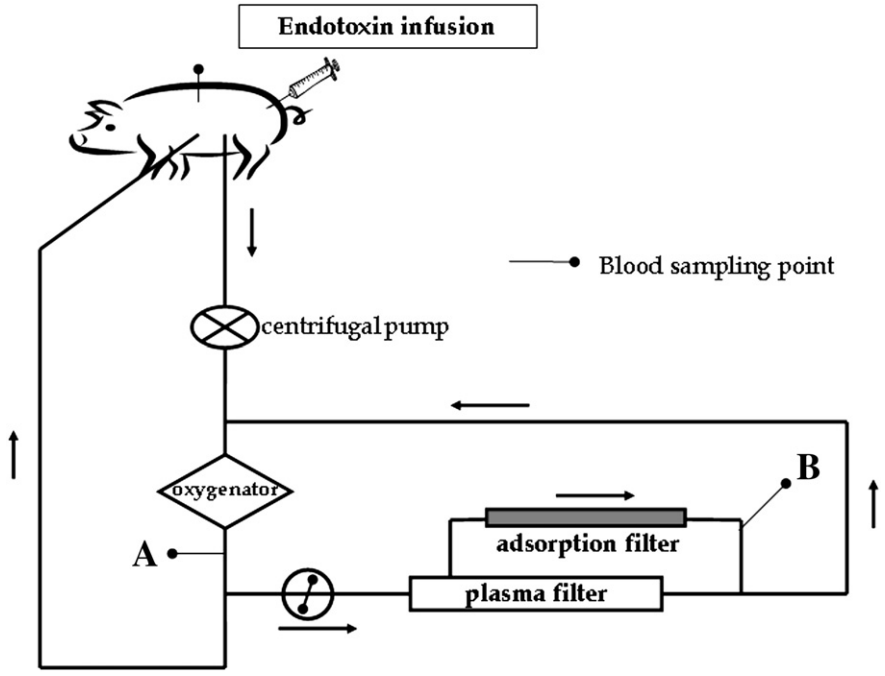

Fig. 1. Experimental setup of the extracorporeal life support (ELS) system and the incorporated resin adsorption circuit. A and B. Blood sampling points before and after the filter.