Work in progress report - Experimental

Partial cardiopulmonary bypass in rats using a hollow fibre oxygenator

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Abstract

Despite new minimally invasive techniques, cardiopulmonary bypass (CPB) is still necessary for many major operations in the field of cardiac surgery. Unwanted side effects of CPB are well known but poorly understood. We therefore developed a rodent model to study the pathophysiology of these potential complications. Male Fischer rats were anaesthetized, intubated and ventilated. The carotid artery and jugular vein were cannulated. The blood was actively drained from the venous circulation and further transferred by a miniaturized roller pump to a hollow fibre oxygenator and back to the animal via the carotid artery. The roller pump produces a pulsatile blood flow between 5 and 40 ml/min. The surface of the hollow fibre oxygenator is 0.025 m². The priming volume (Ringer solution) of the whole system is 12 ml. Animals were catheterized and brought in partial bypass for a mean of 50±15 min. Normal cardiac function after successful weaning was confirmed by electrocardiography and blood pressure measurements. This technical study demonstrates the feasibility of a small animal model of CPB. The main improvement over existing techniques is the use of a highly effective hollow fibre oxygenator with a minimized priming volume. Therefore, no additional animals are needed as blood donors.

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1. Introduction

Since 1970, several attempts to achieve cardiopulmonary bypass in rodents have been described [1–3]. However, these early experiments resulted in pulmonary oedema and cardiac arrest after a relatively short perfusion period [4]. It was, therefore, not possible to establish a decent small animal model for further research. The main problems were of technical nature, as for example the poor stability of the oxygenator or the insufficient venous drainage. Early reports describe models with foam oxygenators, basically bubble oxygenators with direct contact of the gas and blood phase. Therefore, prolonged supportive cardiopulmonary bypass was impossible due to damage to the erythrocytes [5,6], low density lipoproteins [7], and due to recticuloendothelial dysfunction. In 2001, Fabre et al. [8] described a stable model which is relatively easy to handle but blood from additional animals was necessary to prime the system.

A well reproducible model for cardiopulmonary bypass in rats may open the field for various studies on cardiopulmonary bypass and also of systemic ischaemia–reperfusion injury in vivo [9]. The purpose of this series of experiments was to develop a new cardiopulmonary bypass model in rats by the use of a highly effective hollow fibre oxygenator with a very low priming volume.

2. Material and methods

2.1. Animal care

All animals received humane care in compliance with the European Convention of Animal Care formulated by the Swiss Association of Laboratory Animals. The protocol was approved by the local animals study committee.

2.2. Circuit preparation

The extracorporeal bypass model has two main components: a pulsatile roller pump and a hollow fibre oxygenator (Fig. 1) with an integrated heat exchanger, developed and built by our group.
The roller pump consists of a three-head occlusive pump system, which is driven by a direct current motor. It produces a pulsatile blood flow between 5 and 40 ml/min with a range of 100 up to 300 rounds per minute. The stroke volume is between 0.05 and 0.13 ml. The oxygenator is built of a polycarbonate tube (length 130 mm, diameter 5 mm) containing the fibres. At both ends, the fibres are fixed to the tube by two-component glue. The material of the fibres is polypropylene, kindly provided by Dideco, Mirandola, Italy. The housing material is polycarbonate (tube length: 134 mm, outer diameter: 15 mm, inner diameter: 10 mm). The fibres in the oxygenator have an active oxygenation surface of 0.025 m². For cooling and rewarming, a spiral tube with continuous water flow is fixed around the oxygenator tube.

For cannulation, sterile Vygon catheters (14 gauge) were used on the venous and the arterial side. The arterial catheter contains a Teflon tip with an inner diameter of 0.9 mm and an outer diameter of 1.2 mm to maximize blood flow. Venous return is actively drained by the roller pump into a venous reservoir.

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The whole system has a priming volume of 12.0 ml, of which the oxygenator constitutes only 6.2 ml. For priming, Ringer solution is used. No blood from additional animals is needed to prime the system. Oxygen flow through the oxygenator (FiO₂ 1.0) was maintained between 50 and 500 ml/min. At the start of the experiment the oxygen flow was set as high as possible to ensure sufficient oxygenation. The maximum flow was determined in preliminary studies by the observation of bubbles in the blood flow. In subsequent experiments, oxygen flow was reduced to 50 ml/min without any change in oxygen saturation.

2.3. Surgical procedure

Male Fischer F344 rats (270–430 g) were used for the experiments (physiological parameters in Table 1 [10]). The animals were anaesthetized by breathing halothane (4%) in a glass chamber, intubated, and anaesthesia was maintained with halothane. During surgery, the rat was ventilated with a 14-gauge cannula (FiO₂ 1.0, frequency 65, tidal volume 10

Table 1

<table>
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<th>Physiological haemodynamic parameters of rats</th>
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<td>Heart rate (min⁻¹)</td>
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<td>Blood pressure (mmHg)</td>
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<td>Stroke volume (ml)</td>
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<td>Cardiac output (ml/min)</td>
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<td>Blood volume (ml/100 g)</td>
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<td>Respiratory rate (min⁻¹)</td>
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ml/kg body weight) by the use of a Harvard rodent ventilator (Harvard Apparatus, South Natick, MA, USA). During cardiopulmonary bypass, additional halothane was inflated into the oxygenator in order to maintain anaesthesia. Central temperature was measured by a rectal temperature probe and maintained between 35 and 38 °C using a heat exchanger under the operation table and also an integrated heat exchanger in the oxygenator. The femoral artery was cannulated by a 17-gauge catheter (Centractac, Vygon, France) for continuous pressure measurement. The jugular vein was exposed and cannulated with a 14-gauge intravenous catheter (Centractac, Vygon, France). Subsequently, the carotid artery of the opposite side was exposed and cannulated with a 14-gauge intravenous catheter with a Teflon tip placed into the aortic arch. For anticoagulation, heparin (500 IU/kg) was applied, half of the dose directly via the femoral catheter, the other half was given into the extracorporeal circuit.

2.4. Protocol

Before starting the animal experiments, different system parameters such as flow, pressure management and haemolysis were tested in vitro. All data are given as mean ± standard deviation.

A first series of experiments (study 1: n = 6) was conducted on partial cardiac bypass with the miniature roller pump device only. In the following experiments (study 2: n = 6) the hollow fibre oxygenator was added to the circulation system. In all groups a CPB time of 50 min was planned. But due to either technical or surgical problems some animals stayed either longer or shorter on the CPB (mean CPB time: 50 ± 15 min).

2.4.1. Study 1

In this study the biocompatibility of the pump was tested. The animals were monitored by continuous arterial pressure measurement and arterial oxygen saturation. In addition, heart rate and ECG were monitored.

2.4.2. Study 2

In this study, the entire system including the hollow fibre oxygenator was tested for biocompatibility. The same parameters as in study 1 were monitored.
All animals were euthanized in deep anesthesia according to the protocol after re-establishment of spontaneous breathing and haemodynamic stability.

3. Results

The haemolysis of the system was tested under different conditions (Fig. 2). As haemolysis parameter lactate dehydrogenase (LDH) was used. The isolated pump as well as the combination of the pump and the oxygenator were tested at different flow volumes (58 ml/min and 144 ml/min). As control, blood samples were kept on a swinging table (Gasser Apparate & Laborzubehör, Teufen, Switzerland) for the same time interval. No significant haemolysis caused by the pump or by the oxygenator could be detected in the given time frame under physiological flow conditions.

A remarkable haemolysis (LDH > 480 U/l) was detected only when the flow was increased up to 144 ml/min. In all the preliminary experiments the haematocrit and the haemoglobin concentration did not change significantly over the entire observation period.

3.1. Study 1

The pump was tested in six animals. The mean heart rate before connecting the animals to the pump was 294 ± 59 beats/min (Fig. 3) and the mean arterial blood pressure 50 ± 17 mmHg. After connecting the animal to the pump and diluting the animal’s blood with 5.8 ml of Ringer solution from the extracorporeal circuit, the mean heart rate and the mean arterial blood pressure slowed down to 225 ± 55 beats/min and 30 ± 14 mmHg. The animals were perfused by the pump during 50 min. After weaning from the circuit, the heart rate and the blood pressure rose again to 280 ± 28 beats/min and 44 ± 24 mmHg, respectively. The oxygen saturation was between 87 and 97% during the entire observation period. One animal (1/6) died due to arrhythmia during the perfusion with the pump; all other animals recovered uneventfully.

3.2. Study 2

The pump and oxygenator circuit were tested in an additional six animals. The mean heart rate before connecting the animals to the pump and oxygenator was 248 ± 73 beats/min, and the mean arterial blood pressure was 68 ± 21 mmHg. The animals were connected to the system, and the blood of the animals was diluted with 12 ml of Ringer solution. The haemoglobin concentration diminished from 14 ± 1.3 to 8.6 ± 0.9 g/dl. The animals have been supported by the pump and the oxygenator during 50

![Fig. 2. In the physiological range no remarkable haemolysis could be recognized (mean circulating blood volume: 45 ml). Only when the system is driven with high flow could significant haemolysis be detected. LDH standard range: 240–480 U/l.](image2)

![Fig. 3. Reduction of the heart rate and the mean arterial blood pressure during either cardiac or cardiopulmonary bypass and complete restoration of these parameters after weaning from CPB (all groups: n = 6).](image3)
4. Discussion

The aim of this study was to test the feasibility of a new system for cardiopulmonary bypass in rats including a hollow fibre oxygenator. A bypass time of 50 min was chosen because it seemed long enough for surgical procedures in future experiments. To our knowledge, this is the first study mimicking the clinical situation not only with the roller pump but also with a hollow fibre oxygenator.

In the past, few investigators have established CPB in rats. The first model was reported by Subramaniam et al. [1]. In this model, perfusion was established between the right atrium and the femoral artery. The priming volume was as high as 120 ml. Proctor [2] developed an experimental bubble oxygenator with a priming volume of 25 ml. Recently, Fabre et al. [8] evaluated a commercially available hollow fibre oxygenator, which was also used in paediatric cardiac surgery. The priming volume in his studies was 35 ml with the need of additional blood donor animals. In our model, we used a hollow fibre oxygenator, which is a miniature version of the ones used in human cardiac surgery. It was specially tailored for the use in rodents, as we were able to reduce the priming volume to 6.2 ml in the oxygenator. In addition, the very small circulating blood volume, as compared to previously published systems, does not lead to increased haemolysis as assessed by LDH measurements (Fig. 2), underlining the good mechanical properties of this new system.

Because of technical reasons we were not able to measure the in vivo pump output as yet. Therefore, we cannot provide any information about the exact pump flow in vivo. But as shown in Fig. 3, a significant part of the blood volume must have passed the pump, otherwise the decreased heart rate cannot be explained. We can also not quantify the amount of oxygen which was delivered through the oxygenator; however, the oxygen saturation was stable during the experiment. We assume that the bypassed blood was sufficiently saturated with oxygen, otherwise the saturation would decrease significantly during the experiment.

Earlier experimental studies with the aim of achieving experimental CPB have been limited to large animals. This new model is easy to handle and reduces experimental cost and time, and therefore allows performing small animal studies with larger experimental groups. Because the animals tolerate CPB and subsequent weaning very well, this model will also allow the study of the postoperative pathophysiology of CPB and to extend this knowledge with methods of molecular biology. In a first experimental series, for example, it is planned to investigate the influence of hypothermic CPB on intracerebral temperature during cooling and rewarming.

In conclusion, this study demonstrates the technical feasibility of a rodent model for partial cardiopulmonary bypass with an excellent survival rate.

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References