

Research Article

Characterization of an *In Vitro* Model of Extracorporeal Circulation: A New Tool for Investigating the Pathophysiological and Therapeutic Strategies in Whole Blood

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A clinically relevant extracorporeal circulation model would be a valuable tool for investigating the pathophysiological and therapeutic strategies in whole blood. Previous models were limited by issues such as large circuit area; the inability to achieve full bypass; and donor blood requirement for prime. Here we established a miniature circuit to overcome these limitations consisting of a peristaltic pump, a test cell, a blood reservoir, and an oxygenator connected via polyvinylchloride and porous platinum silicon tubing. A heparinised (10 U/mL) saline solution at pH 7.4 was used to prime the circuit and the test cell was incubated in a water bath to maintain the temperature at 37°C. Blood flow through the circuit was at 5 mL/minute rate. Haemodynamics, haemoglobin concentration, and blood gases were analysed and the circuit performance was optimised according to the levels of haemolysis at three circulation time intervals: before the start, 30 minutes, and 60 minutes. No statistically significant haemodynamics and blood gases differences were observed. We have established a miniature extracorporeal circuit consisting of asanguineous prime for CPB model that maintains clinically acceptable results regarding hemodynamic parameters, blood gases, and haemodilution. This surrogate model would be important for further use in clinically pertinent research.

1. Overview

Cardiopulmonary bypass (CPB) is one of the major technological advances in medicine that allows operating in controlled conditions. Since its introduction in the 1950s, there has been a rapid growth in the number of cardiac surgical operations performed throughout the world. Though the mortality for many of these operations has fallen, CPB is still associated with a significant morbidity [1]. CPB is known to activate inflammatory processes that may result in respiratory failure, bleeding disorders, neurologic dysfunction, and transient renal impairment [2]. Furthermore, CPB increases the production of inflammatory cytokines [1] and that cytokines are known to depress cardiac efficiency by mechanisms that are still unclear [3]. Increasing evidence also supports that oxidative stress occurs before the occurrence of proinflammatory factors during cardiopulmonary bypass [4]. This relationship may suggest a direct effect of free radicals on the inflammatory reaction induced by CPB; however, the underlying mechanism of these effects in blood remains elusive. There is a need, therefore, for a preclinical bypass model that could be used to study all aspects of extracorporeal circulating blood pathophysiology and to test novel therapeutic strategies. Previous models [5, 6] that used animal experimental preparations *in vitro* and *in vivo* have been described in the literature; however, the necessary minimization of the circuit gave rise to some obvious problems such as undesirable circuit area, the inability to achieve full bypass, and donor blood requirement for prime. Hence we have established a circuit to overcome these limitations.

2. Description

2.1. The In Vitro Blood Recirculation Model. As shown in Figures 1 and 2, the *in vitro* model consisted of a pump, a test cell, a blood reservoir, and an oxygenator. These



FIGURE 1: Schematic view of the *in vitro* blood recirculation model showing a test cell, blood reservoir, oxygenator, and tubing circuit.

components were connected via polyvinylchloride (PVC) and porous platinum silicon tubing of internal diameter of 1.6 mm. Blood was pumped around the circuit through these tubes using a Watson-Marlow MH101 UR peristaltic pump under flow conditions of 5 mL/minute. The test cell consisted of two acrylic blocks of size $139 \times 110 \times 25$ mm, with an internal compartment of size $89 \times 60 \times 55$ mm, coated with hylosil silicon sealant. Two stainless steel tubes were inserted into the acrylic blocks from opposite ends of the internal compartments to act as inlet and outlet ports. A neoprene rubber gasket surrounded the edges of the internal compartments, which helped to sandwich an ultrafiltration PES50 membrane between the acrylic blocks of the test cell. 12 mL heparinised (10 U/mL) solution composed of 17.5 mL plasmalyte, 12.5 mL gelofusine, 5 mL 10% mannitol, and 1 mL 8.4% sodium bicarbonate, at pH 7.4 was infused into the test cell to prime the circuit, as in clinical CPB. Blood was pumped into the internal compartment via the inlet port, over the ultrafiltration PES50 membrane and then out of the compartment via the outlet port into the oxygenator circuit and reservoir. The reservoir circuit was made up of PVC tubing on the inlet and porous platinum silicon tubing on the outlet that connects it to the oxygenator. The oxygenator was comprised of 3-4 coils of air permeable silicon tubing fitted into a polypropylene vessel that was connected to an oxygen source to provide a maximum surface area for blood oxygenation. The entire circuit was set up in a microbiological class II safety cabinet and the test cell was incubated in a gently rocking water bath to maintain adequate mixing and a blood temperature of 37°C.

2.2. Study Subjects. 50 mL heparinised blood was taken from ischaemic heart disease (IHD) patients (n = 10 per group) and was circulated for 4 hours through our *in vitro* circuit model. The study protocol was approved by the local medical ethics committee and informed consent was obtained from all participating individuals. Analysis of variance for repeated measurements was used to compare the outcome parameters measured in series. All data were presented as mean \pm

 TABLE 1: (a) The effect of flow rate on plasma blood haemoglobin.

 (b) The effect of flow rate on haematocrit.

| (a) | | | | | | |
|-----|--------------------|-------------------|----------|--|--|--|
| | 10 mL/minute | 5 mL/minute | P values | | | |
| 0 | 10.28 ± 2.04 | 10.28 ± 2.04 | N/A | | | |
| 10 | 129.43 ± 16.46 | 14.20 ± 3.54 | < 0.05 | | | |
| 60 | 135.65 ± 40.87 | 32.84 ± 16.36 | < 0.05 | | | |
| 240 | 141.15 ± 29.63 | 60.45 ± 13.69 | < 0.05 | | | |
| | | (b) | | | | |
| | 10 mL/minute | 5 mL/minute | P values | | | |
| 0 | 0.43 ± 0.87 | 0.43 ± 0.87 | N/A | | | |
| 10 | 0.34 ± 0.04 | 0.40 ± 0.03 | < 0.05 | | | |
| 60 | 0.28 ± 0.05 | 0.32 ± 0.11 | < 0.05 | | | |
| 240 | 0.16 ± 0.08 | 0.31 ± 0.10 | < 0.05 | | | |
| | | | | | | |

standard error of the mean. The statistical analysis was performed by using the SPSS software (SPSS Inc., Chicago, IL,USA). Differences were considered significantly at *P* value less than 0.05.

2.3. Optimisation of the Model. Plasma haemoglobin was used as an index of the level of haemolysis induced by flow conditions of the circuit. This was determined using the plasma haemoglobin assay from Sigma Diagnostics, Poole, UK as described previously [7]. The initial model design was optimised according to the levels of haemolysis. The results indicated that a model flow rate of 5 mL/min gave circulating haemoglobin levels of 52.85 \pm 25.35, which was equivalent to that of clinical CPB (46.57 ± 22.45) at 60 minutes of circulation (Tables 1 and 2). Blood samples were collected at different time points: before the start of the pump (time 0), 10 minutes, 30 minutes, and 60 minutes after the start of circulation. A full blood count and blood gases were performed using a SF3000 machine (Sysmex, Japan) and a gas analyser (Ciba-Corning, USA), respectively, to optimise the design of the model (Table 2). When comparing model values to those of clinical CPB in terms of blood gases and blood cell counts no significant difference was observed at flow rate of 5 mL/minute indicating that the model is a good surrogate.

3. Discussion

The use of *in vivo* or *in vitro* experimental models may have advantages and disadvantages, but both are regarded as necessary and complementary. Thus where *in vivo* models are useful to study the physiological relevance and long-term effects of the processes under investigation, by contrast *in vitro* models are not exposed to the internal and external effects, but they are limited by their short duration and stability. This study was carried out using blood from subjects with contrasting clinical outlook in order to test the robustness of the surrogate CPB model on elucidating the way blood responds to stress. The preparation was stable and viable for at least 4 h; the severity of the metabolic acidosis could



FIGURE 2: Overview of the blood recirculation model.

| TABLE 2: Changes w | th time of full blood | l count and gases for mode | el and CPB samples |
|--------------------|-----------------------|----------------------------|--------------------|
|--------------------|-----------------------|----------------------------|--------------------|

| | Sampling time (minutes) | | | | | | |
|-----------------------------------|-------------------------|-------------------|-------------------|------------------|--------------------|-------------------|----|
| | Model | | CPB | | | P values | |
| | 0 | 30 | 60 | 0 | 30 | 60 | |
| PH | 7.45 ± 0.08 | 7.53 ± 0.05 | 7.55 ± 0.01 | 7.41 ± 0.03 | 7.43 ± 0.06 | 7.63 ± 0.04 | NS |
| pCO ₂ | 5.37 ± 0.50 | 3.30 ± 0.44 | 3.00 ± 0.17 | 5.29 ± 0.18 | 4.90 ± 0.36 | 2.95 ± 1.89 | NS |
| pO ₂ | 24.80 ± 5.16 | 27.30 ± 3.76 | 27.93 ± 5.97 | 29.38 ± 0.97 | 23.14 ± 2.88 | 30.24 ± 6.33 | NS |
| Hb (plasma) | 10.87 ± 5.04 | 36.41 ± 36.55 | 52.85 ± 25.35 | 10.58 ± 8.07 | 22.37 ± 7.72 | 46.57 ± 22.45 | NS |
| Hct | 0.33 ± 0.07 | 0.25 ± 0.13 | 0.28 ± 0.11 | 0.26 ± 0.04 | 0.25 ± 0.04 | 0.27 ± 0.04 | NS |
| Platelets counts $\times 10^9$ /L | 217.67 ± 6.66 | 148.33 ± 44.09 | 127.00 ± 20.88 | 180.33 ± 55.64 | 157.33 ± 37.55 | 141.00 ± 36.43 | NS |
| Total WBC $\times 10^9$ /L | 7.33 ± 1.05 | 5.80 ± 0.78 | 4.99 ± 1.02 | 5.05 ± 0.09 | 6.00 ± 1.15 | 5.74 ± 1.65 | NS |

HB: haemoglobin; Hct: haematocrit; WBC: white blood cells count.

be readily evaluated and the success of avoiding haemolysis allowed us to compare the results at before circulation and during the procedure, as is the custom in clinical practice.

Some investigators had previously developed similar models as a primary goal without taking the next step of conducting research to simulate clinical CPB. Our model provides a platform for simulating the effect on blood alone when exposed to artificial surfaces of the extracorporeal circuit and of experimental conditions [8–10]. Utilising this tool, we studied the relationship between the production of oxygen free radicals and the release of proinflammatory cytokines in blood, suggesting that in diseased blood, increased oxygen free radicals may depress and delay production of proinflammatory cytokines by affecting antioxidant activity and complement activation [8, 9]. In these experiments, we observed that the reduction of the foreign surface exposed to the blood significantly reduced the surface activation of blood elements and systemic inflammatory cascades [8, 9]. This allowed us to focus our analysis on blood within the context of the activation of complement that plays an important role in the pathogenesis of ischaemia-reperfusion injury and whether this may be mediated by reactive oxidant species (ROS) [8, 9]. The results from these studies suggested that ROS plays an important role in regulating complement activation independently of the classical and mannan binding lectin (MBL) pathways and that increased activities may be attributable to a direct effect on the alternate pathway [8, 9]. This may occur by direct oxidant modification of complement

proteins such as by nitration and nitrosylation, thus these molecules being less effective in recruiting and stimulating blood leukocytes [10].

The evidence from these studies using this experimental model of blood has limitations which warrant discussion. First the preparation is superfused (surrogate blood circulation) as opposed to being arterially circulated. However, the preclusion of the vasculature as the natural pathway for the provision of substrate may also be advantageous in that confounding effects of the endothelial mediated inflammation and oxidative stress induced by ischaemia and reperfusion are separated. Secondly, although the model can be adapted for use in larger animal studies as it is, it is not appropriate for small animal studies (i.e., mice and rats) without significant modifications in the design.

In conclusion, we have characterised an extracorporeal blood recirculation model that is readily available; the preparation is inexpensive and stable for at least 4 h and above. This permits the researchers to effectively study a host of pathophysiological processes such as the mechanism of generation of oxygen free radicals and their roles in the induction of oxidative stress and proinflammatory factors in a laboratory setup. These types of study could help to establish the identity of the components that play the greatest part in inducing cytokine production and may represent a potential target for the reduction of the inflammatory reaction induced by CPB. In addition, the extended stability of the model may be potentially useful for studying the effect of genetic manipulation on pathophysiological mechanisms underlying injury sustained during ischaemia and reperfusion and developing new therapeutic strategies for combating undesirable effects.

Conflict of Interests

The authors do not have any direct financial relation with the commercial identities mentioned in the paper.

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