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The Effect of Pulsatile Versus Nonpulsatile Blood Flow on Viscoelasticity and Red Blood Cell Aggregation in Extracorporeal Circulation

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Background: Extracorporeal circulation (ECC) can induce alterations in blood viscoelasticity and cause red blood cell (RBC) aggregation. In this study, the authors evaluated the effects of pump flow pulsatility on blood viscoelasticity and RBC aggregation. ***Methods:*** Mongrel dogs were randomly assigned to two groups: a nonpulsatile pump group (n=6) or a pulsatile pump group (n=6). After ECC was started at a pump flow rate of 80 mL/kg/min, cardiac fibrillation was induced. Blood sampling was performed before and at 1, 2, and 3 hours after ECC commencement. To eliminate bias induced by hematocrit and plasma, all blood samples were adjusted to a hematocrit of 45% using baseline plasma. Blood viscoelasticity, plasma viscosity, hematocrit, arterial blood gas analysis, central venous O_2 saturation, and lactate were measured. ***Results:*** The blood viscosity and aggregation index decreased abruptly 1 hour after ECC and then remained low during ECC in both groups, but blood elasticity did not change during ECC. Blood viscosity, blood elasticity, plasma viscosity, and the aggregation index were not significantly different in the groups at any time. Hematocrit decreased abruptly 1 hour after ECC in both groups due to dilution by the priming solution used. ***Conclusion:*** After ECC, blood viscoelasticity and RBC aggregation were not different in the pulsatile and nonpulsatile groups in the adult dog model. Furthermore, pulsatile flow did not have a more harmful effect on blood viscoelasticity or RBC aggregation than nonpulsatile flow.

Key words: 1. Cardiopulmonary bypass
2. Hematology
3. Blood
4. Extracorporeal circulation

INTRODUCTION

Extracorporeal circulation (ECC) causes red blood cells (RBC) and plasma to change in many ways [1-4]. These changes are largely due to the adverse effects of the heart-lung machine caused by non-physiological contact between blood and artificial surfaces [1], the effects of mechanical shear stress on blood cells [2], associated hemodilution [3], and nonpulsatile

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perfusion flow [4]. In particular, these effects of EEC on RBCs and plasma cause alterations of hemorheology, such as blood viscoelasticity and RBC aggregation. Blood viscoelasticity is an intrinsic property that governs flow throughout the circulatory system in response to pressure developed by the heart [5] and is affected by hematocrit (Hct), temperature, plasma viscosity, RBC deformability, and aggregation tendency [6,7]. RBC aggregation is mainly determined by plasma protein composition and the surface properties of RBCs, which change according to plasma concentrations of acute phase reactants in inflammatory disorders [8]. Furthermore, RBC aggregation is known to fall during cardiopulmonary bypass (CPB) because of hemodilution [9,10].

The comparative effects of pulsatile and nonpulsatile flow in regard to blood trauma remain debatable [11], and studies concluding that blood trauma is related to flow type have generally focused only on hemolysis. In this study, we investigated whether pulsatile perfusion has more harmful effects on blood viscoelasticity and RBC aggregation than nonpulsatile perfusion.

METHODS

1) Anesthesia and surgical method

All animals were treated according to the Guide for the Care and Use of Laboratory Animals issued by the Korea University School of Medicine. Twelve mongrel dogs, weighing 27.24±5.20 kg (range, 23–32 kg), were randomly assigned to a nonpulsatile group (NP group, n=6) or a pulsatile group (P group, n=6). Animals were premedicated with intramuscular ketamine (10 mg/kg) and placed on a surgery table after weighing. An intravenous fluid route was established at the upper foreleg. After inducing general anesthesia with thiopental sodium (5–10 mg/kg) and vecuronium bromide (0.1 mg/kg), a 6–7 French endotracheal tube was inserted, and anesthesia was maintained using a N_2O/O_2 gas mixture (2 L/min of each). Mechanical ventilation was maintained at a tidal volume of 10–15 mL/kg and a respiratory rate of 20–25 breaths/min.

To monitor hemodynamic data and to obtain blood samples, a 20 G catheter containing a pressure transducer was inserted through the right femoral artery and a 16 G catheter was inserted through the right femoral vein for venous blood gas analysis.

Median sternotomy was performed in the supine position, heparin (3 mg/kg) was injected, the ascending aorta was cannulated (18 Fr), and the right atrium was subjected to bicaval cannulation (22 Fr). These cannula were then connected to a nonpulsatile centrifugal pump (Bio-Source TM200; Biomedicus, Minneapolis, MN, USA) or a pulsatile pump (T-PLS, Twin-Pulse Life Support, SL-1000; NewheartBio Co., Seoul, Korea). The extracorporeal circuit was primed with lactated Ringer's solution. A membrane oxygenator (CAPIOX SX10R Oxygenator; Terumo Medical Co., Ann Arbor, MI, USA) was used in both groups. After measuring baseline hemodynamic parameters and blood sampling, ventricular fibrillation was induced with a 9 V battery. ECC was started at pump flow rate of 80 mL/kg/min. Body temperatures were maintained at 36°C. Hemodynamic parameters and blood sampling were performed using the same methods at baseline and 1, 2, and 3 hours after ECC commencement. At the end of the experiment, animals were euthanized in an anesthetized state.

2) Sample preparation for viscoelasticity and aggregation index measurements

At baseline (before ECC), 30 mL of arterial blood was collected from the right femoral artery, and after 1, 2, and 3 hours of EEC, 15 mL of arterial blood was extracted. These samples were kept in iced water before separating RBCs and plasma using a centrifugal separator at 2,500 rpm for 10 minutes. To eliminate bias caused by Hct and plasma, all blood samples were adjusted to a Hct of 45% using baseline plasma.

3) Plasma viscosity and blood viscoelasticity measurements

Plasma viscosity and blood viscoelasticities were measured using a Thermo Scientific HAAKE MARS rheometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 36°C. The test unit waited for 1 minute before measuring viscosity and viscoelasticity values to allow blood to achieve a quiescent state. Sample plasma viscosity and blood viscoelasticity values were measured using a frequency of 1 Hz and logarithmically spaced shear strains ranging from 10 to 80 s^{-1} (shear rate 62.2, 75.5, 91.4, 110.8, 134.1, 162.6, 197.0, 238.6, 289.1, 350.2, 424.1,

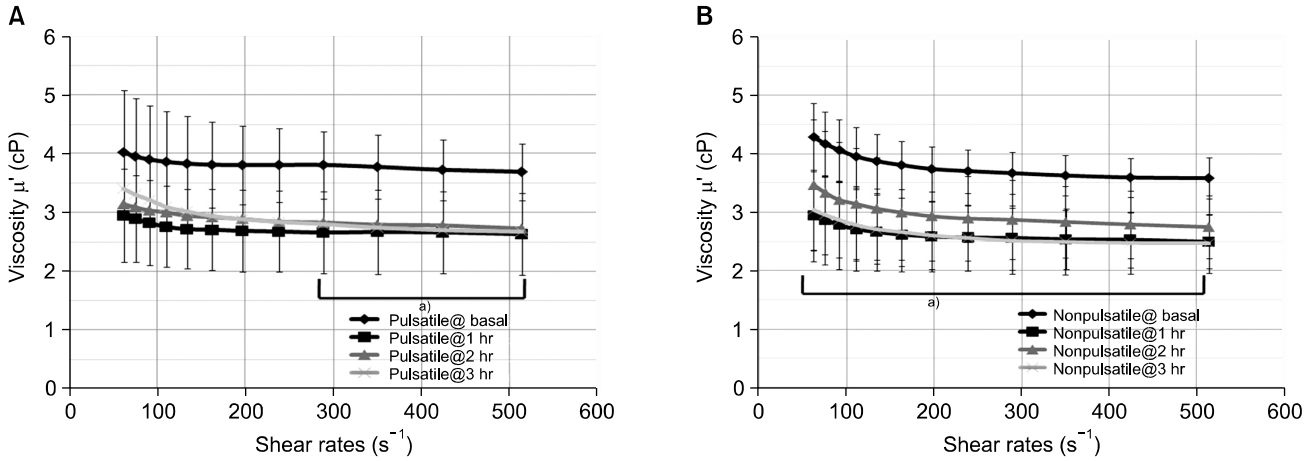

Fig. 1. (A) Viscosity changes of pulsatile group. (B) Viscosity change of nonpulsatile group. The viscosity of the adjusted whole blood is shown in shear rates ranging from 62.2 to 513.9 s^{-1} in logarithmic space. There was no difference between the two groups, but the viscosity of the two groups decreased abruptly 1 hour after ECC and remained low during ECC. ECC, extracorporeal circulation; cP, centipoise. [a]$p<0.05$ between basal and 1 hour.

and 513.9 s^{-1}). The viscoelastic properties of a fluid can be calculated using the equation.

μ=viscoelastic properties of a fluid= $\mu' - i\mu''$ [5]
μ'=viscosity, μ''=elasticity

4) Aggregation index

A microfluidic device was developed for measuring static aggregation. The device was composed of a NEMESYS syringe pump (Cetoni GmbH, Korbussen, Germany), a 532 nm lase pointer (LuckyDuck; Leadlight Technology Inc., Tao-Yuan, Taiwan) as a light source, a photomultiplier tube (PMT; Dongwoo Optron, Gwangju, Korea) as a detector, and a height gauge (Mitutoyo, Tokyo, Japan) to control the laser pointer. Signals were acquired using a data acquisition (DAQ) board and LabView (National Instruments Inc., Austin, TX, USA). Because flow rates were lower at the periphery of the PMT flow stream, flow in the measurement area was almost stopped for measurements. The infusion and withdrawal profile of the syringe pump was adjusted to disaggregate whole blood cells at rest and to quickly stop the flow stream in a short period of time. The zero flow condition was verified by examining flow streams under a microscope. During this stoppage, blood flow could not move into the large volume at the side, and the rest of the blood tended to move into the main channel. This reduced noise-induced problems owing to the uninterrupted flow. This setup made it possible to measure the static aggregation index (AI). The light emitted by the laser pointer was transmitted to the PMT through whole blood cells, and electrical signals produced by the PMT were transferred from the DAQ board to the LabView program. AIs during quiescent flow were calculated using data for 100 seconds based on the completed time of the withdrawal period for a syringe pump, as previously described [12].

5) Blood test

Arterial blood gas analysis, venous blood gas analysis, lactate, and Hct were measured at baseline and after 1, 2, and 3 hours of ECC.

6) Statistical analysis

The Mann-Whitney test was used to analyze differences between groups. Longitudinal comparisons within groups were performed using Kruskal-Wallis variance analysis followed by Dunn's test. The analysis was conducted using SPSS ver. 10.0 for Windows (SPSS Inc., Chicago, IL, USA), and statistical significance was accepted for p-values <0.05. Results are expressed as means±standard deviations.

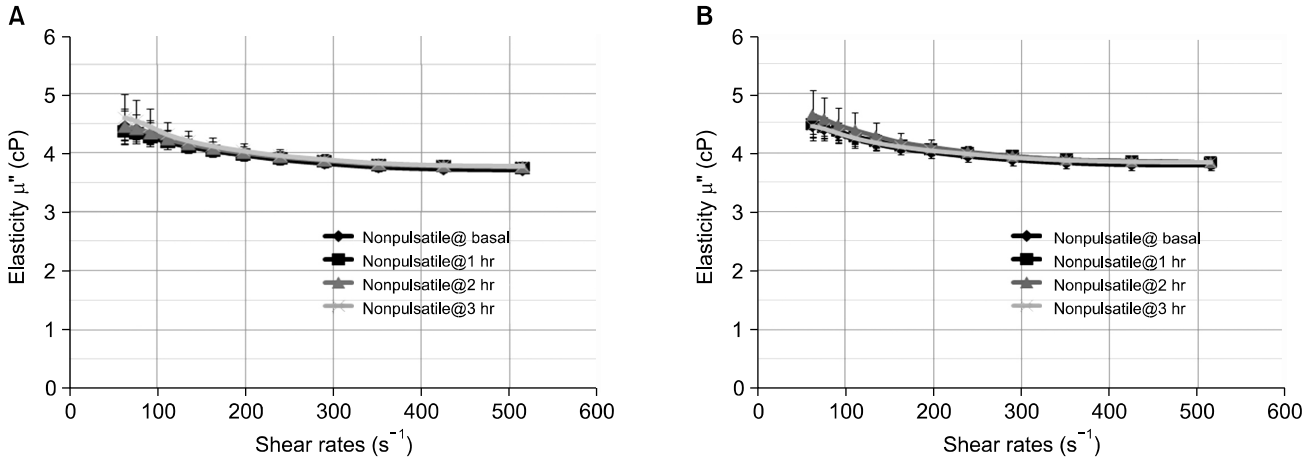

Fig. 2. Elasticity. There was no difference between the two groups. cP, centipoise.

RESULTS

Body weights (NP, 26.0±3.1 kg; P, 25.7±2.3 kg; p=0.833) and total amounts of Ringer's solution infused (NP, 5,433.3±1,645.2 mL; P, 6,183.3±2,139.5 mL; p=0.512) were similar in the P and NP groups.

1) Blood viscoelasticity

Blood viscoelasticity has two components: viscosity and elasticity. Blood viscosity decreased abruptly after 1 hour of ECC and then remained low. However, no significant intergroup differences were observed (Fig. 1). Blood viscosity at baseline was significantly higher than at 1 hour (at 289.1 and 513.9 s^{-1}) in the P group, and baseline blood viscosity was significantly higher at all shear rates in the NP group (Fig. 1). Blood elasticity was constant during ECC in both groups and no significant intergroup difference was observed (Fig. 2).

2) Aggregation index

Mean AI values abruptly decreased after 1 hour of ECC in both groups, and no significantly intergroup difference was found.

3) Hematocrit, plasma viscosity, lactate, and central venous oxygen saturation

Hct and plasma viscosity decreased abruptly after 1 hour of ECC and then remained low during ECC ($p<0.05$). However, no significant intergroup difference was observed (Table 1). Lactate was significantly higher and $ScvO_2$ (central venous oxygen saturation) was significantly lower at 1 hour in both groups ($p<0.05$), but again no significant intergroup difference was evident (Table 1).

DISCUSSION

ECC causes RBC damage, the best-known example of which is hemolysis. However, ECC also induces sub-hemolytic blood traumas, such as RBC aggregation and blood viscoelasticity changes [13]. In the present study, RBC aggregation decreased significantly during ECC, and it has been previously reported that RBC aggregation decreases after CPB because of hemodilution [9,10]. This has been shown to be primarily due to decreases in fibrinogen concentration in plasma [9,10]. Unlike previous studies, we adjusted all blood samples used for aggregation measurement to a Hct level of 45% using baseline plasma, and thus the decreased aggregation observed in the present study was not due to hemodilution only. Furthermore, there was no difference in the AI between the P and NP groups, which suggests that perfusion type has little effect on RBC aggregation.

Blood viscosity decreased during ECC regardless of perfusion type in the present study with no intergroup difference. Blood elasticity remained constant during ECC in both groups and, no elasticity difference was observed between the two groups. Undar et al. [14] reported that viscosity and elasticity changes induced by pulsatile flow during ECC were

Table 1. Changes in laboratory results

Variable	Group	Basal	1 Hour	2 Hours	3 Hours	p-value[a]
PO_2	NP	143.8±70.8[c]	269.8±69.2[d]	263.2±85.2[d]	271.3±78.6[d]	0.02
	P	206.7±95.7	226.5±61.6	235.7±70.3	250.2±106.9	0.85
	p-value[b]	0.24	0.31	0.94	0.82	
Hematocrit (%)	NP	34.4±4.5[c]	19.6±1.8[d]	18±4.2[d]	16.6±4.2[d]	<0.001
	P	32.6±2.5[c]	16.7±3.7[d]	16±4.9[d]	13.9±4.6[d]	<0.001
	p-value[b]	0.38	0.13	0.38	0.42	
Lactate (mmol/L)	NP	3.6±1.1[c]	9.3±0.7[d]	9.6±1.8[d]	11.4±3.2[d]	<0.001
	P	2.5±1.7[c]	7.8±2.9[d]	10.2±2.6[d,e]	12.3±2.8[e]	<0.001
	p-value[b]	0.23	0.47	0.93	0.75	
Central venous oxygen saturation (%)	NP	51.3±17.4[c]	32.5±10.8[d]	18.7±4.3[e]	19.2±5.9[e]	<0.001
	P	52.5±15.4[c]	35.2±19.2[d]	22.5±11.9[d]	26±4[d]	0.01
	p-value[b]	1.0	1.0	0.33	0.08	
Plasma viscosity (cP)	NP	1.4±0.1[c]	1.2±0.1[d]	1.1±0.1[d]	1.2±0.1[d]	<0.001
	P	1.5±0.3	1.2±0.1	1.3±0.2	1.2±0.2	0.08
	p-value[b]	0.38	0.34	0.34	0.42	
Aggregation index (%)	NP	49.33±3.93[c]	37.83±10.98[d]	32.67±5.85[d]	29.67±6.12[d]	0.001
	P	54.0±0.97[c]	37.33±11.50[d]	33.67±12.45[d]	32.67±13.01[d]	0.02
	p-value[b]	0.38	1.0	0.94	0.75	

Values are presented as mean±standard deviation.
NP, nonpulsatile group; P, pulsatile group.
[a]The significances of longitudinal intragroup differences were determined using the Kruskal-Wallis test. [b]The significances of intergroup differences were determined using the Mann-Whitney test. [c,d,e]Same letters indicate non-significant differences between groups as determined using Dunn's test.

less than those induced by nonpulsatile flow during deep hypothermic circulatory arrest. Blood viscoelasticity depends on a number of factors, such as Hct, temperature, plasma viscosity, RBC deformability, aggregation tendency, and plasma composition [5-7]. We believe that the different results obtained by Undar et al. [14] and ourselves were caused by the use of different methods of measuring viscoelasticity and temperature during ECC. We conducted ECC at 36°C, whereas Undar et al. [14] measured viscoelasticity under hypothermic conditions. In addition, Undar et al. [14] did not adjust for Hct level or fix the plasma protein level. Furthermore, they used a neonatal piglet model, while we used an adult dog model. RBC aggregation and plasma viscosity are known to be lower in infants than in adults [15]. Despite the debate regarding the superiority of pulsatile flow with respect to organ protection during ECC in adults, general agreement has been reached that pulsatile flow is beneficial in pediatric patients [16]. Thus, it appears that the protective effect of pulsatile flow on blood cells might differ in pediatric and adult patients. Nonetheless, in the present study, we did not find any significant viscoelasticity difference between pulsatile and nonpulsatile flow during ECC in an adult dog model.

This study has some limitations that warrant consideration. First, the number of study subjects was small. Second, excessive hemodilution caused by circuit priming could have induced tissue hypoxia, generated oxygen free radicals, and thus adversely affected RBCs [17-23] and masked the effects of flow type on blood viscoelasticity and RBC aggregation. Third, RBC aggregation during ECC does not precisely reflect *in vivo* RBC aggregation, because of the adjustment made for Hct and plasma composition. Accordingly, we suggest more studies be conducted on a larger number of animals to confirm the effect of pulsatile flow on blood viscoelasticity and RBC aggregation in adults.

In summary, we found no difference between pulsatile and nonpulsatile flow with respect to RBC aggregation or blood viscoelasticity in an adult ECC model.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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