UNIVERSITY OF CALIFORNIA, SAN DIEGO

Effects of Viscogenic Plasma Expander on Cardiac and Vascular Function

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy

in

Bioengineering

by

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2010

The Dissertation of Surapong Chatpun is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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DEDICATION

To my Parents and Family

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FIELDS OF STUDY

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Studies in Microhemodynamics and Microciculation
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ABSTRACT OF THE DISSERTATION

Effects of Viscogenic Plasma Expander on Cardiac and Vascular Function

by

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Lowering the amount of red blood cells (RBCs) caused by blood losses or anemia leads to the reduction of tissue oxygenation and anaerobic metabolism, ultimately inducing multi-organ dysfunction. Plasma expanders (PEs) and blood are used sequentially to treat volume deficit. Recently, the systemic and microscopic recovery has been proposed to be related to the restoration of the viscosity of circulating blood to values prevailing during normovolemic conditions. Studies in an awake hamster window chamber showed that the viscosity of PE plays a role in microvascular function. High viscosity PEs (HVPEs) have been tested in conditions of hemorrhagic shock resuscitation

and acute hemodilution, supporting the contention that the restoration of blood viscosity to normal value is beneficial in studies made in awake animals. Recent studies, in awake animals, have shown that polyethylene glycol conjugated to human serum albumin (PEG-HSA) can achieve the similar results caused by HVPEs. However, the effects of increasing plasma viscosity on the heart performance are insufficiently explored.

The hypothesis of this study was that cardiac and vascular function is enhanced when plasma viscosity is increased even though the amount of RBCs decreases. Hemorrhagic shock resuscitation and acute hemodilution protocols were performed to test this hypothesis. Cardiac function was assessed in the left ventricle with a pressure-volume conductance catheter. As the modulation of plasma viscosity, the effects of shear stress-induced vasodilator, nitric oxide (NO), on cardiac function was examined. Furthermore, the effect of interaction of PE with endothelial glycocalyx on cardiac function was investigated.

The results showed that HVPE and PEG-HSA demonstrated the beneficial effects on cardiac function in acute hemodilution by increased cardiac output, lowered systemic vascular resistance and sustained cardiac contractility. However, HVPE did not show any enhancement on cardiac function in hemorrhagic shock resuscitation. There was a contribution of increased NO to the beneficial effects on cardiac function as observed in the acute hemodilution with low viscosity PE. It also revealed that PEG-HSA and HVPE interacted with endothelial glycocalyx with different mechanism but they showed similar results in cardiac function, implying an important role of endothelial glycocalyx in cardiac and vascular function.

Chapter 1

General Introduction

The reduction of the amount of red blood cells (RBCs) in pathological conditions, for example hemorrhagic shock and anemia, lowers tissue oxygenation, leads to anaerobic metabolism and ultimately induces multi-organ dysfunction. In case of severe blood loss, blood or fluid infusions are used to treat volume deficit. Plasma expanders (PEs) are infused fluids which restitute blood volume by expanding the volume of plasma. Regarding to increasing blood volume with PEs, transfusion trigger and oxygen delivery criteria have been studied and investigated for decades [3, 5, 7, 12, 35, 40]. Recent studies have shown that hematocrit (Hct) can be lowered beyond the conventional transfusion trigger (21% for Hct)[7, 13]. Furthermore, modulating plasma viscosity with PEs shows the enhancement of blood circulation and the maintenance of tissue perfusion after hemorrhagic shock resuscitation and extreme hemodilution in awake animals [10, 12, 35, 40]. Therefore, the viscosity of PEs plays an important role in the recovery of blood flow and tissue perfusion.

Viscosity of Plasma Expanders

Blood is a non-Newtonian fluid whose viscosity varies with shear rate and depends on plasma viscosity and the concentration of RBCs. Normal blood plasma viscosity is about 1.0-1.2 cP and the viscosity of PEs varies over a broad range from 1 to 8 cP. The viscosity of PEs can be enhanced by either using large molecular species or increasing the number of solute molecules. However, using large molecular species can cause coagulation disorders and renal function impairment, while increasing the number

of solute molecules can lead to high osmotic pressure. These biological limitations should be considered when the viscosity of PEs is modulated.

To study how the viscosity of PEs plays a role in the blood circulation, numerous experiments have been performed in animals such as dog, rat, hamster and mouse. Two strategies often used to investigate the effects of plasma viscosity on the circulatory system, especially microcirculation, are hemodilution and hemorrhagic shock resuscitation models. In animal studies, a functional capillary density (FCD), the length of capillaries which RBCs flow through in a field of view over observation time, is used to assess microvascular perfusion. Early studies in the awake hamster window chamber model revealed that FCD can be maintained or restored by increasing plasma viscosity after hemorrhagic shock resuscitation and blood transfusion in extreme hemodilution [4, 5, 8, 10, 12, 13, 40]. Systemic parameters also showed significantly improved after high viscosity plasma expanders (HVPEs) infusion in a hemorrhagic shock resuscitation model [11, 40]. Microhemodynamic parameters such as arteriolar velocity and arterial blood flow were also enhanced after resuscitation and hemodilution using HVPEs compared with low viscosity plasma PEs (LVPEs) [5, 10, 11, 35, 40]. In addition, the results of these studies demonstrated that infusion or exchange with HVPEs can prolong favorable microhemodynamic conditions better than exchange with LVPEs. These findings emerge from studies in systemic circulation and microvasculature, without consideration of heart function.

There is the potential that viscosity of PEs influences on the pumping performance of the heart. Wettstein et al. found that cardiac index, the ratio between cardiac output and animal weight, improved after volume resuscitation with HVPE (20%

hydroxyl ethyl starch; HES 20%) when compared with LVPE (HES 5%) [40]. Furthermore, they showed that the recovery of capillary perfusion significantly improved with HES 10% and HES 20% compared with HES 5%. As blood perfusion improved and maintained nearly the baseline condition after resuscitation with HVPE, it may imply that cardiac function was also restored. Therefore, understanding how the viscosity of PEs affects cardiac function is important.

Cardiac Function Assessment

The pumping performance of the heart can be assessed by many cardiac function indices such as cardiac contractility, cardiac output, stroke volume and stroke work. Techniques such as biplane fluorography, thermodilution, flow probe, micromanometer, conductance catheter and echocardiography have been used to assess cardiac function in normal and pathological stages of animals [1, 20, 23, 24, 39, 42].

Studies in anesthetized large animals investigated the effects of infusion fluids such as hypertonic saline and dextran 70 kDa, on the cardiac function after shock resuscitation. Ogino and coworkers introduced a micromanometer pressure transducer into left ventricle combining with an ultrasonic transit-time flowmeter probe placed around the root of ascending aorta to study cardiac function [28, 29]. They did not find any significant differences in the cardiac contractility and the cardiac diastolic function after hemorrhagic shock between the group using normal saline and the group using hypertonic saline and dextran70 (HSD). This finding was similar to Welte's work that HSD did not increase cardiac contractile function during small volume resuscitation from hemorrhagic shock in anesthetized pigs [38]. Despite studies of Ogino et al. and Welte et al., Horton and colleagues resuscitated with HSD plus lactated Ringer's solution in burn

shock guinea pigs and reported that this solution could enhance the contractile performance of the heart [21]. Unfortunately, how viscosity of infused fluid affects cardiac function was not considered in these studies. Therefore, a role of plasma expander viscosity on cardiac function is still in question and becomes a substantial topic to investigate.

The recent developments of miniaturized pressure-volume (PV) catheter provide continuous real time measurement for pressure, volume and the derivatives, high temporal resolution and more insight to cardiac function of small animals through pressure-volume analysis for each cardiac cycle [18, 23, 30, 31]. The PV catheter consists of two excitation electrodes generating an electrical field inside the cardiac chamber, two sensing electrodes measuring a voltage change which is proportional to a change in resistance and a pressure sensor in the middle of catheter's length. The conductance signal of the PV catheter is itself noncalibrated and needs to be converted to absolute volume. The primary equation relating conductance to volume is $V = 1/\alpha (\rho L^2)(G–G_p)$, where ρ is the blood resistivity, L is the distance between sensing electrodes, G is the measured conductance, G_p is the parallel conductance due to conductivity of the muscle wall and surrounding tissues and α is a gain coefficient (volume correction/calibration factor). Generally, there are two approaches to calibrate and convert the measured conductance to actual volume. One is to use a series of known-volume cylinders with blood to put the PV catheter to measure conductivity and create a a conductance-to-volume relationship. The other approach to calibrate the PV catheter is to assess cardiac output or stroke volume to calculate α using other measuring techniques

such as Doppler flow probe, volume flow probe and echocardiography. The parallel conductance can be determined from hypertonic saline calibration described by Baan and colleagues [2]. Recently, a novel technique to measure the parallel conductance has been proposed by Wei et al. using admittance and Wei's equation [33, 36]. In general, the PV catheter has an advantage over other available approaches to measure cardiac function because it independently measures cardiac function from loading condition and heart rate and provides a real-time measurement in both pressure and volume.

Shear Stress-induced Nitric Oxide and Vascular Mechanotransduction

Changes in blood viscosity in vivo may be related to changes in vascular geometry due to autoregulatory process driven by changes in the production of endothelium-derived relaxation factors. The concepts of mechanotransduction were introduced and considered as an important mechanism of flow regulation in microcirculation [16, 17]. These concepts were further investigated by Tsai et al. in the hamster skinfold model to relate with plasma viscosity, shear stress-dependent release of vasoactive mediator-nitric oxide (NO) and functional capillary density [35]. They found HVPE increased greater wall shear stress compared to LVPE in the acute hemodilution model. Increasing wall shear stress enhances the arteriolar and venular blood flow in the microcirculation. Furthermore, HVPE replacement in moderate hemodilution increased organ blood flow which could not be observed with LVPE [15, 19]. Many studies have revealed that the endothelium-derived nitric oxide depends on the wall shear stress which directly regulates the activity of nitric oxide synthase (eNOS) [9, 16, 25, 41]. Theoretically, for Newtonian fluid, wall shear stress (τ) is a function of fluid velocity (u) and viscosity (μ), in a term of $\tau = \mu(du/dy)$. Therefore, changing in blood viscosity can

affect the NO synthesis on the endothelial cells. Kimura et al. increased the shear stress in the coronary circulation by adding three different concentrations of dextran 40 kDa into the perfusion fluid in isolated perfused rat hearts [25]. They found that coronary blood flow decreased by 30% in the first few minutes, and it gradually increased and was steady for 10 minutes in a group added with 0.5% dextran. Moreover, when they gave N^G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide inhibitor, in the perfusion fluid, the coronary flow was lower than the control group which has no L-NAME. Regarding to their study, it showed that the introduction of high viscosity solution plays a role in the regulation of coronary flow due to increase in NO production. In addition, NO-induced vasomotion affects the local blood flow distribution, due to blood viscosity change, in hemorrhagic shock resuscitation and hemodilution. Generally, in cardiovascular system, there is a coupling between a heart and a vascular system. Therefore, the performance of a heart may be affected by changing in a local blood flow distribution regarding to NO production from the blood shear stress stimulation. However, there is inadequate understanding about the role of shear-induced NO released by endothelial cells on the cardiac function.

Recently, it has been proposed that the endothelial glycocalyx is a mechanosensor in transducing fluid shear stress into intracellular cytoskeleton of endothelial cells, leading to the intracellular signaling activation and the production of vasoregulatory agents, mainly NO [22, 26]. Glycocalyx is an important thin layer lined on the surface of endothelium, which functions as a barrier between endothelial cell and flowing blood [25, 27, 37]. Glycocalyx is a 3D meshwork of proteoglycans, glycoproteins, glycosaminoglycans and adsorbed plasma proteins. The study by Pahakis et al. showed

that there is the interrelationship between the glycocalyx and NO production [32]. Using enzymes i.e. heparinase, chondroitinase, neuraminidase and hyaluronidase which selectively degrade the endothelial glycocalyx components demonstrated that there was attenuation in NO production. Furthermore, Cabrales et al. intravenously injected a bolus of Streptomyces hyaluronidase in an awake hamster window chamber model to study the effects of endothelial glycocalyx on microhemodynamic [14]. They reported that there was no significant difference in vascular tone, velocity and blood flow after the enzyme injection. However, they found a decrease in functional capillary density in a group treated with the degradation enzyme. Therefore, they suggested that the decrease in glycocalyx leads to capillary perfusion impairments due to FCD reduction. Another study showed increasing permeability of water, albumin and HES after ischemia in isolated guinea pig hearts when heparinase was introduced [34]. These findings demonstrated that endothelial glycocalyx was not only a transducer of shear force but it also influenced extravasations of infusion solution and fluid filtration. Recently, Jacob et al. have performed the experiment in guinea pig isolated hearts using Krebs-Henseleit buffer added with 1/3 volume 5% human albumin or 6% HES [22]. Their study has revealed that coronary flow in albumin group increased about double compared with the HES group even though the solution viscosity of albumin was lower than that of HES. They have concluded that albumin induces greater endothelial shear stress than HES, despite its lower viscosity. This finding implied that albumin has the potential effect on glycocalyx, leading to an increase of NO synthesis much better than HES. However, there is still insufficient knowledge about the interaction between PEs and endothelial glycocalyx and the role of endothelial glycocalyx in vasoactivity and cardiac function.

Aims and Approaches

This study proposed that the treatment of a severe reduction in the hematocrit can be aided by the use of viscogenic agents to restitute the circulating blood viscosity, leading to the beneficial effects on cardiac and vascular function. Three specific aims were proposed and experimentally investigated.

Specific Aim 1

Previous studies in awake hamsters have focused on elucidating local microvascular phenomena and mechanisms related to the changes in plasma viscosity as a consequence of introduction of the PEs. Nevertheless, the conventional microvascular response may not sufficiently provide the answers to identify the cause that leads to the enhanced microvascular function when plasma viscosity is elevated. According to tissue perfusion was improved and maintained after resuscitation with HVPEs and PEG-HSA in shock protocol and after exchange with these fluids in hemodilution protocol, the blood perfusion in the heart may follow this phenomenon. In addition, this phenomenon may lead to enhance the performance of the heart after treatment with HVPEs and PEG-HSA in the pathological protocols. Therefore, it is significant to determine that cardiac function after hemorrhagic shock resuscitation and acute hemodilution with viscogenic PEs exhibits improvements related to the findings in the microcirculation of the awake hamster window chamber model.

To attain this aim, the PV catheter was introduced to assessed left ventricular function in acute hemorrhagic shock resuscitation and isovolemic hemodilution with different viscosity of PEs in anesthetized hamster.

Specific Aim 2

As increased plasma viscosity during hemorrhagic shock resuscitation and hemodilution in an awake hamster model, enhanced microvascular function has been observed. Increased plasma viscosity leads to a change in shear stress on the vascular wall and a release of vasomediators, mainly NO. Previous study found that NO concentration increased after resuscitation with Dex500 (HVPE) [35]. There may have interesting mechanisms between the NO production and cardiac function. Therefore, it is important to investigate the contribution of shear stress-induced NO on cardiac function.

To achieve this aim, DETA NONOate (NO donor) and vehicle were administered to anesthetized hamsters during isovolemic hemodilution with low viscosity PE.

Specific Aim 3

Previous works in awake animals have shown that PEG-HSA effectively maintained microvascular function without significantly increasing plasma viscosity or shear stress, while HVPE markedly increased plasma viscosity [6, 10, 12, 13]. These finding implied a different interaction with vasculature between PEG-HSA and HVPE. Furthermore, it may deliberately relate to the property and structure of PEG-HSA that interact with endothelial glycocalyx, a shear stress mechanosensor, in vasculature. Therefore, it is substantial to investigate the contribution of endothelial glycocalyx in cardiac function and to determine that the mechanism of mechanotransduction by PEG-HSA is mediated by its interaction with endothelial glycocalyx.

To accomplish this aim, Streptomyces hyaluronidase, a hyaluronic acid specific enzyme, will be introduced to degrade hyaluronic acid, a component of glycocalyx, in anesthetized hamster undergoes hemodilution with PEG-HSA and HVPE.

Understanding the effect of viscogenic PEs on cardiac function in a specific aim1 shows how the heart works when plasma viscosity changes and may in part explain the observation in the awake hamster window chamber model that microvascular function can be restored in shock resuscitation and hemodilution experiments after transfusion with HVPE and PEG-HSA. In specific aim2, the results provide information how NO synthesized from endothelial cells affects on cardiac function when plasma viscosity is changed. Specific aim3 demonstrates how the plasma expander-endothelial glycocalyx interaction couples with cardiac and vascular function. This may provide information why PEG-HSA and HVPE showed similar beneficial effects in the systemic and microvascular function, although they had markedly different viscosity.

Organization of the Text

This chapter provides a brief introductory of viscosity of plasma expander. We discussed previous works in microvascular study that demonstrated the beneficial effects of increased plasma viscosity in pathological conditions such as hemorrhagic shock resuscitation and acute anemia. Furthermore, a recent novel measurement technique to assess cardiac function was briefly explained. A review of shear stress-induced NO and vascular mechanotransduction was provided to explain how HVPEs demonstrated beneficial effects in microvascular function and what a possible reason causing PEG-HSA evidenced similar results showed by HVPEs. We further discussed our study aims and approaches to achieve the aims.

Chapter 2, chapter 3 and chapter 4 describe the effect of PE viscosity on cardiac function as an achievement of specific aim 1. Chapter 2 specifically describes the

assessment of left ventricular function in the sodium pentobarbital-anesthetized hemorrhagic shock model. Chapter 3 and chapter 4 explain the effects of Viscogenic plasma expanders on cardiac function using moderate hemorrhagic shock-resuscitation and acute hemodilution models, respectively. We observed that PEG-HSA efficiently improved cardiac output and preload and lowered pumping work per stroke volume during hemodilution as similar results as HVPE. In addition, PEG-HSA provided beneficial effects on cardiac function in hemorrhagic shock resuscitation over HVPE.

Chapter 5 describes the effects of increased vascular NO on cardiac function in acute hemodilution with LVPE as an accomplishment of specific aim 2. We observed that exogenous NO released by NO donor provided the beneficial effects on ventricular function by increase in cardiac output, reduction of systemic vascular resistance and lower in stroke work per ejected volume, in an acute hemodilution with LVPE.

Chapter 6 describes the role of endothelial glycocalyx integrity on cardiac function during acute hemodilution with PEG-HSA and HVPE that related to specific aim 3. It demonstrated that glycocalyx degradation caused fluid leakage to extravascular tissues. In acute hemodilution, we found that hyaluronic acid might interact with PEG-HSA but not with HVPE, causing a deteriorated cardiac function.

Chapter 7 concludes all important findings in this study related to the specific aims. Strengths and weaknesses of this study and future works are also suggested.

Appendix I and II provide supplement on a mathematical relationship between stroke work and fluid viscosity and left ventricular volume estimation, respectively.

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Chapter 2

The Assessment of Left Ventricular Cardiac Function in the Sodium Pentobarbital-Anesthetized Hemorrhagic Shock Model

Abstract

Hemorrhagic shock results from massive losses of intravascular volume of blood, leading to hypotension, anaerobic metabolisms, ischemia, heart failure, multi-organ dysfunction and eventually death. Early studies have suggested that cardiac failure was a result of lowering in oxygen availability and impairment of myocardial contractile function when hemorrhagic shock was profound. Measurement and analysis of cardiac function *in vivo* can be complex and challenged during hemorrhagic shock, especially in small animals. Furthermore, most cardiac function measurements require animals under anesthesia. In general, anesthetics are cardiodepressive agents. Sodium pentobarbital is a commonly used as a general anesthetic for cardiovascular physiological studies in experimental animals. Therefore, pentobarbital-anesthetized animals under hemorrhagic shock condition may have more depressive effect on cardiac function. This study aimed to characterize the effect of sodium pentobarbital on cardiac function and to assess cardiac function in hamster subjected to a hemorrhage of 40% of blood volume. Cardiac function were measured and evaluated by a pressure-volume conductance catheter. Two groups of animals were performed; one group was under anesthetic without hemorrhage as a control group and other group was under anesthetic with hemorrhagic shock. Mean arterial pressure (MAP) in animals with hemorrhagic shock decreased to 50% of baseline while animals without hemorrhage had MAP at 80% of baseline. The maximum rate of

pressure change (dP/dt_{max}) was 77% and 68% of baseline in the control and hemorrhagic shock groups, respectively. An estimated left ventricular end-systolic elastance in the hemorrhagic shock group was higher compared with the control group ($p<0.05$), implying the compensatory mechanism by enhancing contractile function to increase blood pressure and blood perfusion. Cardiac output, stroke volume and stroke work profoundly decreased after hemorrhage and significant difference compared with the control group ($p<0.01$). Animals anesthetized with sodium pentobarbital evidently depressed on cardiac function in both systole and diastole and the effect severely becomes depressive during hemorrhagic shock.

Keywords: hemorrhagic shock; pentobarbital; cardiac function; conductance catheter

Introduction

Hemorrhagic shock is a pathological state with insufficient blood volume and low arterial blood pressure in a circulatory network, leading to hypoperfusion, low oxygen delivery and anaerobic metabolism. Hemorrhagic shock may effectively affect the pumping performance of the heart and finally cause cardiac dysfunction. In vivo studies in animals have demonstrated that cardiac contraction is depressed during hemorrhagic shock [1, 8, 9, 13, 14, 17]. Several evaluation techniques such as cardiac output curve, pressure-volume relationship and indices of systolic and diastolic function have been used to characterize and assess heart function during hemorrhagic shock [1, 4, 5, 13, 25]. However, measurement and assessment of cardiac function in small animals are more complex and challenged during hemorrhagic shock.

To measure and assess cardiac function, animals need to be maintained under anesthesia. Most of anesthetics are cardiodepressants which have significant effects on cardiovascular activities [10, 22, 24, 26]. Animals with shock condition may be more sensitive to the cardiodepressive effects of anesthetics. Sodium pentobarbital is a commonly used as a general anesthetic agent for cardiovascular physiological study in experimental animals. Without shock condition, cardiac function was profoundly altered and decreased with sodium pentobarbital compared with other anesthetics such as isoflurane, a mixture of ketamine and xylazine [16, 19, 26].

The purpose of this study was to characterize the effect of sodium pentobarbital on cardiac function and to assess cardiac function during the acute moderate hemorrhagic shock in hamster. We applied a miniaturized conductance catheter to measure left ventricular pressure and volume in the fixed volume hemorrhagic shock protocol. The

indices of systolic and diastolic function were derived from the pressure-volume measurements to quantify and assess cardiac performance between with and without hemorrhagic shock conditions.

Materials and Methods

Animal preparation

Studies were performed in anesthetized male Golden Syrian hamsters (Charles River Laboratories, Boston, MA) weighing 60-70g. Animal handling and care followed the NIH Guide for Care and Use of Laboratory Animals. The experimental protocol was approved by the local animal care committee. Animals were anesthetized with sodium pentobarbital (50mg/kg, i.p.) to perform surgery. The left jugular vein and left femoral artery were catheterized for fluid infusion, blood pressure monitoring and blood withdrawal. Tracheotomy was performed and cannulated with a polyethylene-90 tube to facilitate spontaneous breathing. Animals were put in the supine position on the heating pad to maintain the body temperature. During experiment, if animals response to a toe pinching, a small bolus of sodium pentobarbital (10-15mg/kg, i.p.) will be given.

Inclusion criteria

Animals under anesthesia were suitable for the experiments if animals had no surgical bleeding and systemic parameters were within the normal range, namely, mean arterial blood pressure (MAP) above 80 mmHg, heart rate (HR) above 320 beats/minute and systemic hematocrit (Hct) above 45%.

Systemic parameters

The MAP and HR were monitored continuously (MP150, Biopac System Inc., Santa Barbara, CA), except when blood was sampled for laboratory parameters and blood conductance calibration and withdrawn for hemorrhage. The Hct was determined from centrifuged arterial blood samples taken in heparinized capillary tubes. Hemoglobin (Hb) content was measured by spectrophotometer (B-Hemoglobin, Hemocue, Stockholm, Sweden).

Blood chemistry

Arterial blood was collected in a heparinized capillary tube (0.05ml) and immediately analyzed for arterial oxygen partial pressure (PaO_2), arterial carbon dioxide partial pressure ($PaCO_2$), base excess (BE) and pH (Blood Chemistry Analyzer 248, Bayer, Norwood, MA).

Cardiac function

The closed chest method was performed to assess cardiac function in this study. The right common carotid artery was exposed allowing a 1.4F pressure-volume conductance catheter (PV catheter; SPR-839, Millar Instruments, TX) to be inserted. The PV catheter was advanced passing through the aortic valve into the left ventricle [16]. Parallel volumes (V_p) at the baseline, 15 min after hemorrhage and the of shock period were determined by a small bolus intravenous injection of 15% hypertonic saline (10μl). The V_p during the shock period was assumed to be similar to that determined at the end of shock period. The pressure and volume signals were instantaneously digitized and acquired (MPVS300, Millar Instruments, TX and PowerLab 8/30, ADInstruments, CO).

Estimation of left ventricular blood volume

To determine the blood volume in the left ventricle, it is necessary to convert the measured blood conductance (RVU: relative volume unit) to the actual blood volume (μl). Arterial blood was sampled 0.15 ml for the blood conductance calibration at the baseline and the end of shock. The calibration of conductance catheter was performed using a series of 4 known-volume cylindrical cuvettes (14.14, 22.09, 31.81 and 43.30 μl). Blood volume measured by a conductance catheter was determined by $V_{lv} = S * RVU + C - V_p$; where V_{lv} is the absolute left ventricle volume, RVU is the blood conductance measured by PV catheter, S and C are the slope and the y-intercept of linear regression from blood calibration, respectively, as demonstrated in Figureure 2.1 and V_p is the parallel volume caused by the tissues surrounding left ventricle.

Estimation of end-systolic elastance

The left ventricular end-systolic elastance (E_{es}) was estimated using the bilinearly approximated time-varying elastance curve from the selected single beat [20]. This approximation basically considered pressure values, systolic time interval, end-systolic volume and stroke volume during isovolumic contraction and ejection phases.

Moderate hemorrhagic shock protocol

Anesthetized animals were withdrawn 40% of estimated blood volume (BV) via the femoral artery catheter within 15 minutes to induce a moderate hemorrhage. The total BV was estimated as 7% of body weight. The shock condition was conducted for 90 minutes. Systemic parameters (MAP, HR, Hb, Hct and blood chemistry) were measured and analyzed as schematically shown in Figure 2.2. Animals were maintained under anesthesia over the time of experiment.

Experimental groups

Before the experiment, animals were randomly divided into the 2 groups: Sham Control (SC; n=5) without hemorrhagic shock and Hemorrhagic Shock (HS; n=5) without resuscitation.

Data analysis

Cardiac function data were analyzed with PVAN software (version3.6, Millar Instruments, TX). Indices of systolic and diastolic function were calculated including maximum rate of pressure change (dP/dt_{max}), ejection fraction (EF), left ventricular end-systolic pressure (P_{es}), minimum rate of pressure change (dP/dt_{min}), ratio between dP/dt_{max} and end-diastolic volume ($dP/dt_{max}/V_{ed}$), left ventricular relaxation time constant (Tau), maximum filling volume rate (dV/dt_{max}) and left ventricular end-diastolic pressure (P_{ed}). Other cardiac function indices such as cardiac out (CO), stroke work (SW) and stroke volume (SV) were also considered. In addition, stroke work was normalized by stroke volume (SW/SV) representing the work done by a heart per unit volume (SW/SV). The values of studied cardiac function indices were averaged from selected 8-12 cardiac cycles at each time point. The parameters were analyzed before hemorrhage (baseline) and after hemorrhage (shock).

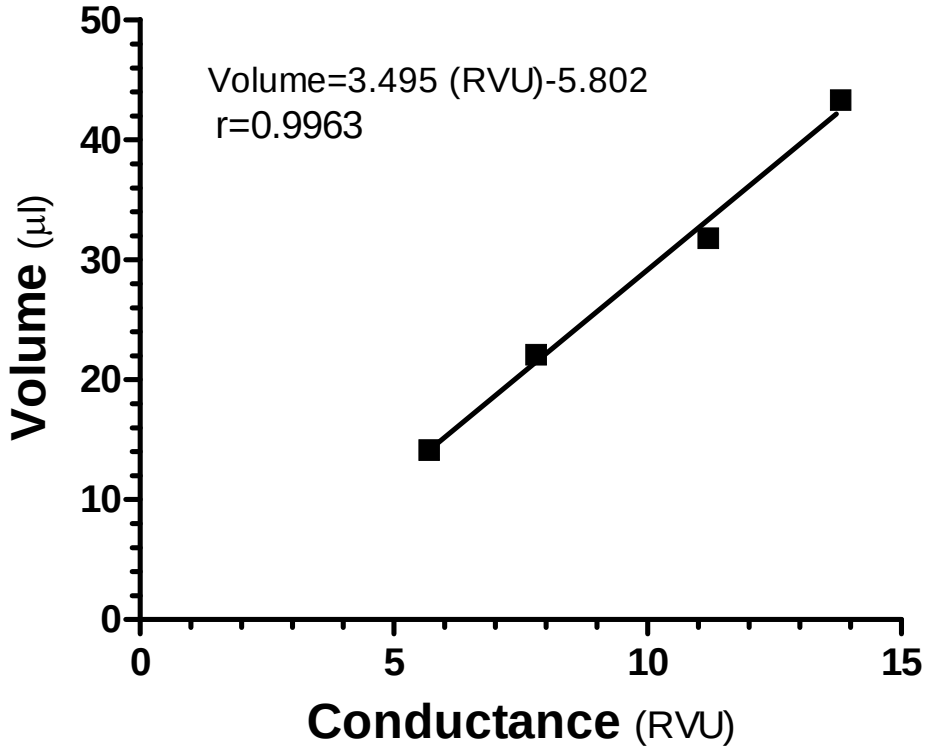

Figure 2.1: Linear regression of blood calibration from blood conductance (RVU) to actual volume (µl) in each known-volume cylindrical cuvette.

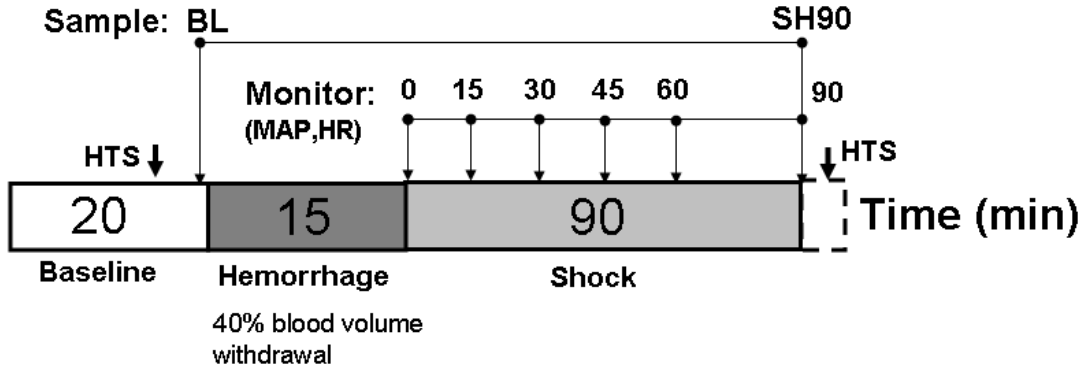

Figure 2.2: Schematic diagram of fixed volume-hemorrhagic shock protocol. BL, baseline; SH90, 90 minutes after beginning of shock; HTS, hypertonic saline; MAP, mean arterial pressure; HR, heart rate.

Statistical analysis

Results are presented as mean ± standard deviation (SD) unless otherwise noted. The values are mostly presented as relative to level at baseline. A ratio of 1.0 signifies no change from baseline, whereas lower or higher ratios are indicative of changes proportionally lower or higher than baseline. Data between interested time points in a same group were analyzed using analysis of variance for repeated measures (ANOVA) and followed by post hoc analyses with the Tukey's multiple comparison tests. Two-way ANOVA test with a post hoc t test with Bonferroni correction for multiple comparisons was used to compare between groups at time point of interest. All statistics were calculated using GraphPad Prism 4.01 (GraphPad Software, San Diego, CA). Results were considered statistically significant if $p<0.05$.

Results

Systemic parameters

Table 2.1 presents systemic and blood gas parameters measured at the baseline and the end of shock period (SH90). There was no significant difference in systemic and blood gas parameters at the baseline between two groups. As expected, pH, BE, Hct, and Hb significantly decreased after hemorrhage due to acidosis and blood loss in the HS group compared with the SC group ($p<0.01$). There was apparently increased in PaO_2 ($p<0.01$) but not significantly different in $PaCO_2$ between groups.

Mean arterial pressure showed a significant decrease after hemorrhage compared to baseline as demonstrated in Figure 2.3 ($p<0.001$). During the early phase of shock (from SH0 to SH30), MAP continually increased and gradually dropped over the time

until the end of observation. At the end of experiment, MAP was about 50% of baseline and animals still survived in the HS group. Interestingly, MAP in the SC group also gradually decreased over the observation period. The MAP in the SC group at the end of experiment was about 80% of baseline and significantly dropped relative to baseline ($p<0.01$). The significant difference between groups in MAP was observed over the shock period ($p<0.001$). On the other hand, there was no significant difference between groups in HR. However, HR in the SC group slightly increased but HR in the HS group gradually decreased over the time as shown in Figure 2.4.

Table 2.1: Laboratory parameters

Parameters	Sham Control (SC) Group	Hemorrhagic Shock (HS) Group	Sham Control (SC) Group	Hemorrhagic Shock (HS) Group
	Baseline		SH90	
PaO_2, mmHg	44.9 (6.0)	48.5 (4.9)	52.9 (14.4)	87.3 (20.3) ††
$PaCO_2$, mmHg	46.2 (6.8)	45.5 (8.6)	37.9 (6.8)	32.9 (4.5)
Arterial pH	7.34 (0.03)	7.34 (0.07)	7.41 (0.05)	7.11 (0.11) †††
BE, mmol/l	-1.6 (2.2)	-1.6 (3.7)	-1.2 (2.3)	-17.9 (4.1) †††
Hct, %	53 (2)	49 (3)	52 (1)	30 (4) †††
Hb, g/dl	16.0 (0.8)	14.7 (1.3)	16.1 (0.4)	9.1 (0.8) †††

Values are means (SD). PaO_2, arterial partial O_2 pressure; $PaCO_2$, arterial partial CO_2 pressure; BE, base excess; Hct, systemic hematocrit; Hb, hemoglobin content of blood. †† $p<0.01$; ††† $p<0.001$ between groups.

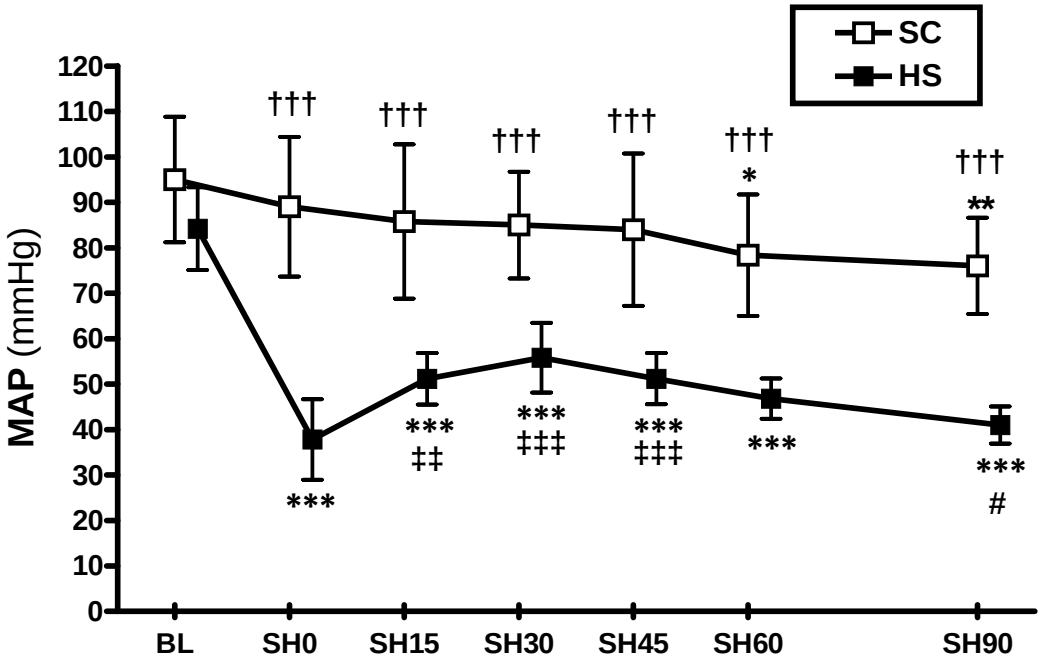

Figure 2.3: Mean arterial pressure (MAP) measured at baseline (BL), at beginning of shock (SH0), at 15, 30, 45, 60 and 90 minutes after beginning of shock (SH15, SH30, SH45, SH60, SH90). Values are presented as means ± SD. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared with baseline. ‡‡ $p<0.01$; ‡‡‡ $p<0.001$ compared with SH0. # $p<0.05$ compared with SH30. ††† $p<0.001$ between groups.

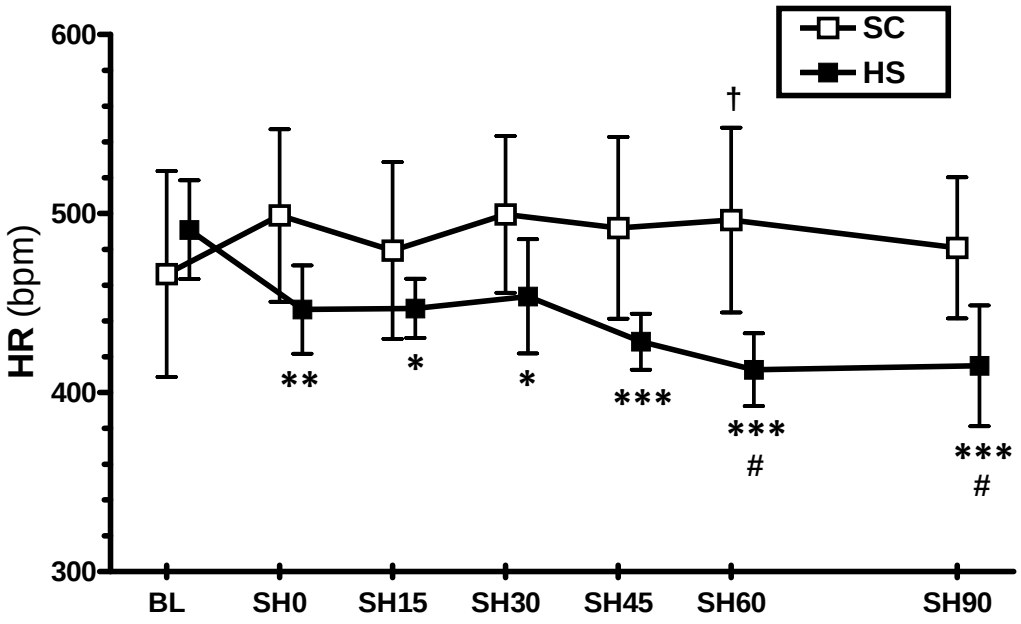

Figure 2.4: Heart rate (HR) measured at baseline (BL), at beginning of shock (SH0), at 15, 30, 45, 60 and 90 minutes after beginning of shock (SH15, SH30, SH45, SH60, SH90). Values are presented as means ± SD. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared with baseline. # $p<0.05$ compared with SH30. † $p<0.05$ between groups.

Left ventricular systolic indices

The dP/dt_{max}, the load-dependent contractility index, in both groups gradually decreased over the observation time in a similar direction as illustrated in Figure 2.5A. At the end of experiment (SH90), dP/dt_{max} was 77% and 68% of baseline in the SC and HS groups, respectively. However, there was not significantly different in dP/dt_{max} between the SC and HS groups. In the SC group, EF did not change from the baseline level, while it was higher than baseline level in the HS group (Figure 2.5B). Although there was a difference in EF between two groups, it was not statistically significant. At the late phases of shock (SH60 and SH90), the decrease of end-systolic pressure (P_{es}) in the SC group was significantly pronounced relative to baseline as presented in Figure 2.5C ($p<0.01$). On the other hand, P_{es} in the HS group did not significantly change relative to baseline. Figure 2.5D presents that the ratio of dP/dt_{max} and V_{ed} in the HS group dropped after SH30 to the end of experiment but it was still higher than baseline. In contrast, this ratio in the SC group was lower than baseline and it significantly decreased relative to baseline at the end of shock period ($p<0.05$). The $dP/dt_{max}/V_{ed}$ showed a significant difference between groups at 30min after hemorrhage ($p<0.05$).

Left ventricular end-systolic elastance

Figure 2.6 presents the approximated left ventricular end-systolic elastance (E_{es}) during hemorrhagic shock. Animals in the SC group showed a significant decrease in E_{es} relative to baseline during hemorrhagic shock ($p<0.05$). The significant difference between groups was found at 30 and 60 min after hemorrhage (SH30 and SH60, $p<0.05$). Figure 2.7 shows the linear regression between $dP/dt_{max}/V_{ed}$ and E_{es} for both the SC and HS groups. It was found that $dP/dt_{max}/V_{ed}$ had a linear relationship with E_{es} (E_{es}=0.83

$dP/dt_{max}/V_{ed}$ + 0.18, r=0.55 for the SC group and E_{es}=0.55 $dP/dt_{max}/V_{ed}$ + 0.28, r=0.69 for the HS group).

Left ventricular diastolic indices

The dP/dt_{min} significantly decreased from baseline over the time in the HS group as shown in Figure 2.8A (p<0.001). In contrast, animals in the SC group maintained dP/dt_{min} at the baseline level. However, dP/dt_{min} was significantly different between groups over the shock period (p<0.01). Figure 2.8B clearly shows that animals under hemorrhagic shock had a higher relaxation time constant (Tau) than animals without hemorrhagic shock due to less blood volume. At the late phases of shock, Tau in the HS group was significantly higher than baseline (p<0.05). Furthermore, at 60 min after hemorrhage, there was a significant difference in Tau between groups (p<0.05). The dV/dt_{max} was also derived to evaluate the diastolic function of the heart. Regarding to a reduction in blood volume, dV/dt_{max} in the HS group dropped significantly compared with baseline (Figure 2.8C; p<0.001). The difference of dV/dt_{max} between groups was also significant for all time points of interest (p<0.001). No statistically significant change in P_{ed} relative to baseline was found in both the SC and HS groups. There was not significant between groups in P_{ed} after hemorrhage.

Cardiac function parameters

Figure 2.9A shows the CO during hemorrhagic shock. Animals in the SC group maintained CO slightly higher than the baseline level for the entire observation time. On the other hand, animals in the HS group had a significantly lower CO relative to baseline and the SC group (p<0.001). The CO in the HS group essentially dropped to 60% of baseline during a shock period.

The SW in the SC group was lower than the baseline level during a shock phase as demonstrated in Figure 2.9B. As expected, animals in the HS group showed a significant decrease in SW as a result of blood loss ($p<0.001$). The SC group performed a stroke work significantly higher than the HS group ($p<0.01$). It was also found that SV in the HS group was significantly lower than that in the SC group (Figure 2.9C; $p<0.01$). As similar as SW, the significant reduction in SV was caused by blood loss during hemorrhage. However, when we considered SW/SV, it was interesting that this ratio was maintained over the time of observation in the SC group but not in the HS group (Figure 2.9D). Despite the reduction of SW/SV in the HS group, there was no significant difference in SW/SV comparing between two groups.

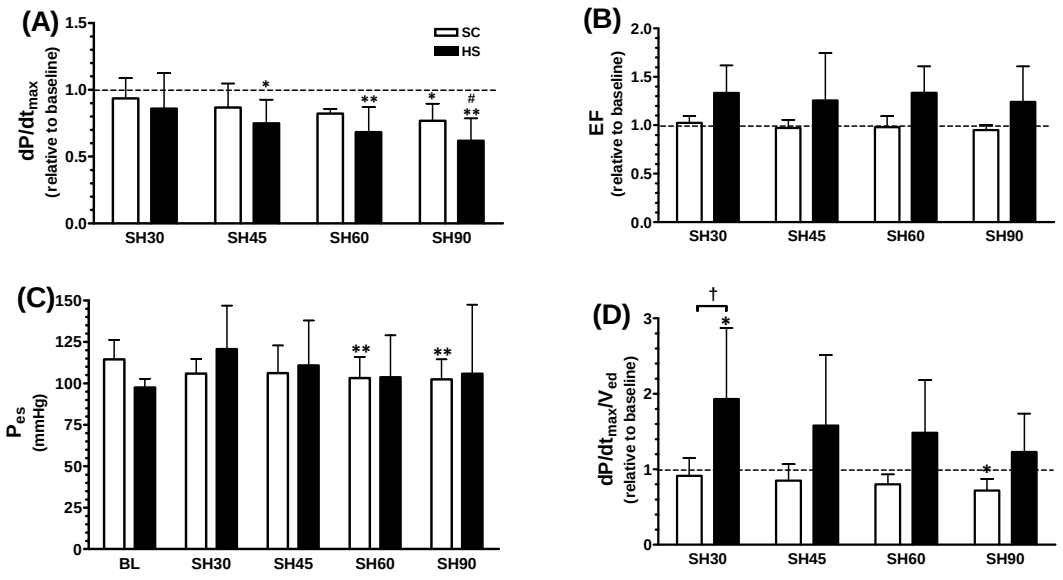

Figure 2.5: Left ventricular systolic function indices derived by PV conductance catheter. (A) Maximum rate of pressure change (dP/dt_{max}) during hemorrhagic shock. (B) Ejection fraction (EF) during hemorrhagic shock. (C) Left ventricular end-systolic pressure (P_{es}) at baseline and during hemorrhagic shock. (D) Ratio of maximum rate of pressure change to end-diastolic volume ($dP/dt_{max}/V_{ed}$) during hemorrhagic shock. Broken line represents the baseline level. Values are presented as means ± SD. * $p<0.05$; ** $p<0.01$ compared with baseline. # $p<0.05$ compared with SH30. † $p<0.05$ between groups. Time points as in Figure 2.3.

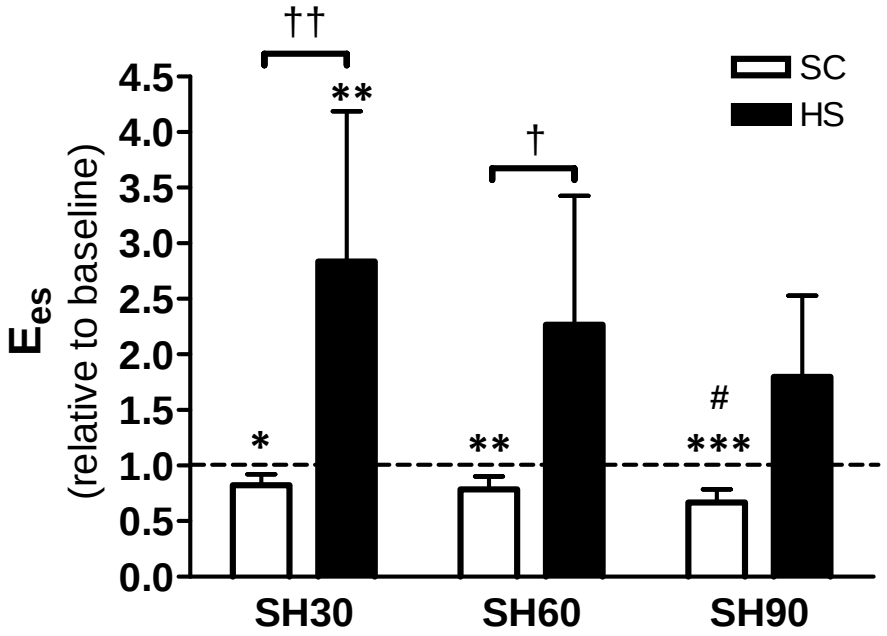

Figure 2.6: Approximated left ventricular end-systolic elastance (E_{es}) during hemorrhagic shock. Broken line represents the baseline level. Values are presented as means ± SD. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared with baseline. # $p<0.05$ compared with SH30. † $p<0.05$; †† $p<0.01$ between groups. Time points as in Figure 2.3.

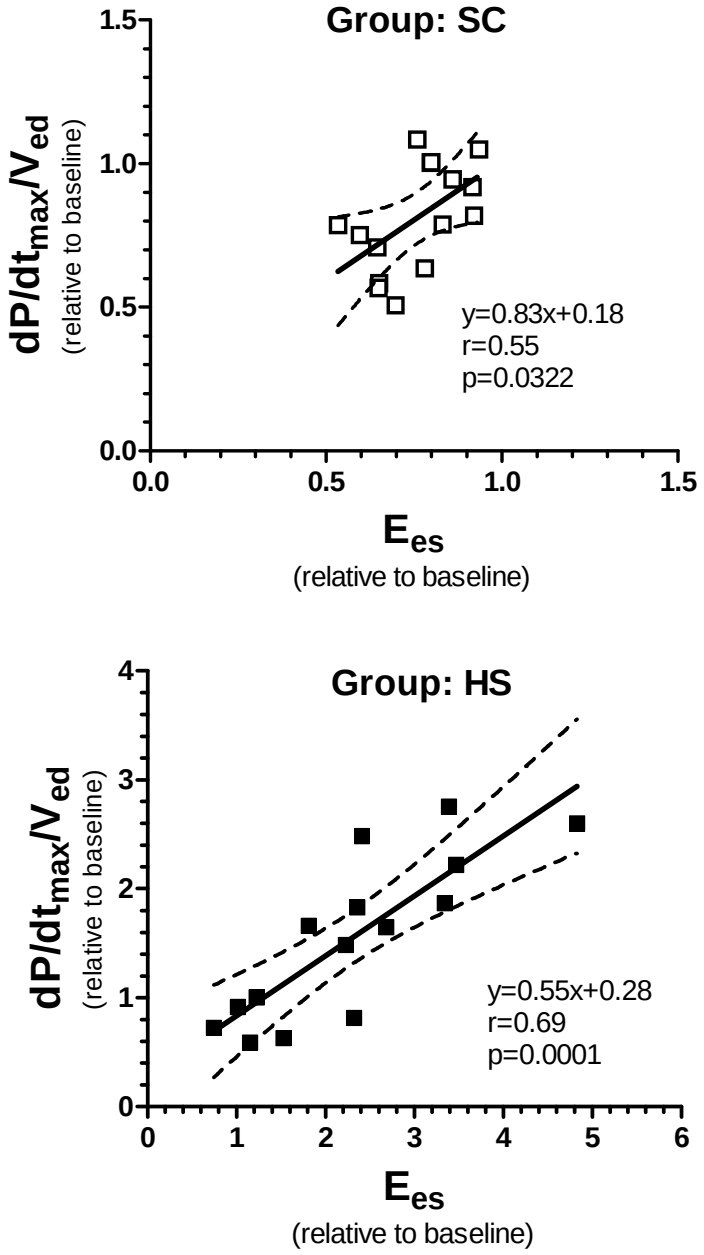

Figure 2.7: Linear regression between $dP/dt_{max}/V_{ed}$ and the estimated end-systolic elastance (E_{es}) with 95% confidence of interval (n=15 for each group).

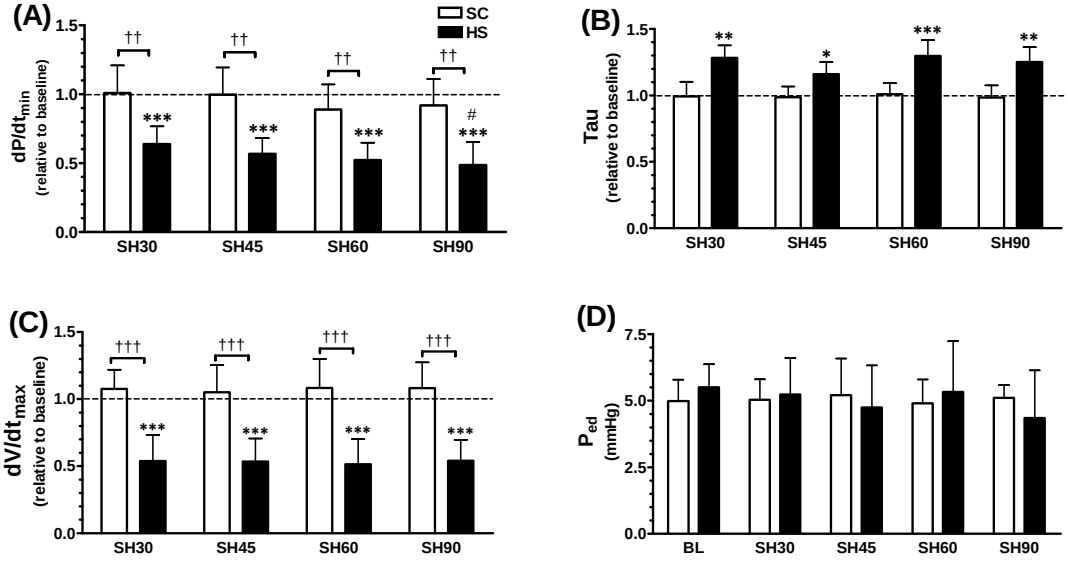

Figure 2.8: Left ventricular diastolic function indices derived by PV conductance catheter. (A) Minimum rate of pressure change (dP/dt_{min}) during hemorrhagic shock. (B) Relaxation time constant (Tau) during hemorrhagic shock. (C) Maximum filling volume rate (dV/dt_{max}) during hemorrhagic shock. (D) Left ventricular end-diastolic pressure (P_{ed}) at baseline and during hemorrhagic shock. Broken line represents the baseline level. Values are presented as means ± SD. * p<0.05; ** p<0.01; *** p<0.001 compared with baseline. # p<0.05 compared with SH30. †† p<0.01; ††† p<0.001 between groups. Time points as in Figure 2.3.

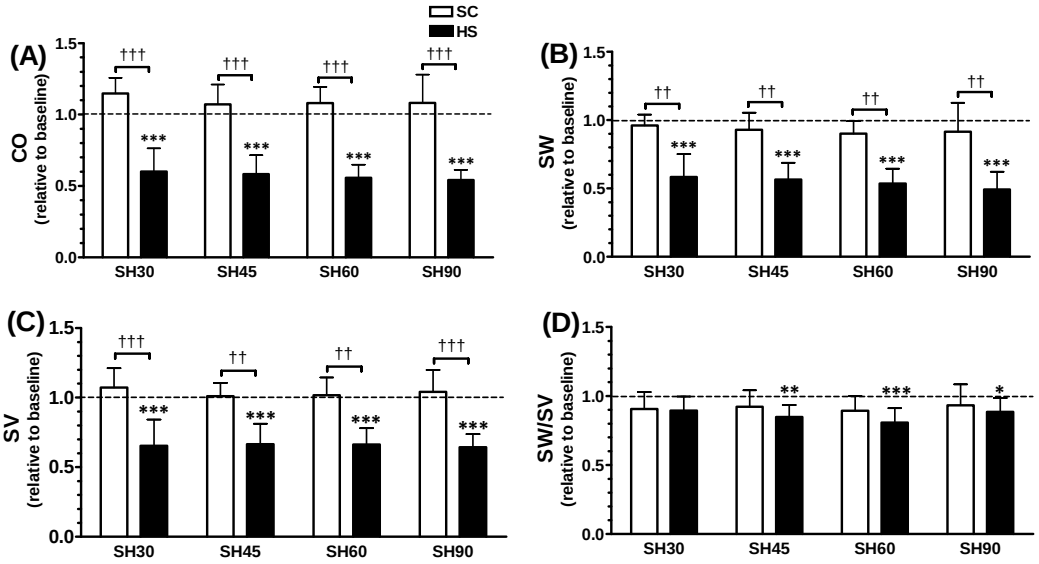

Figure 2.9: Other left ventricular cardiac function indices derived by PV conductance catheter. (A) Cardiac output (CO) during hemorrhagic shock. (B) Stroke work (SW) during hemorrhagic shock. (C) Stroke volume (SV) during hemorrhagic shock. (D) Work done per stroke volume (SW/SV) during hemorrhagic shock. Broken line represents the baseline level. Values are presented as means ± SD. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared with baseline. †† $p<0.01$; ††† $p<0.001$ between groups. Time points as in Figure 2.3.

Discussions

The principal finding of this study is that the depression of left ventricular function, especially systolic function, in anesthetized animals without hemorrhagic shock was caused by sodium pentobarbital. The cardiac contractility was enhanced during the early stage of hemorrhagic shock as illustrated by an increase in E_{es} and $dP/dt_{max}/V_{ed}$. Our results further suggest that hemorrhagic shock effectively caused a deficit in diastolic function and a reduction in load-dependent parameters such as CO, SW, SV and dP/dt_{max}.

Sodium pentobarbital (PB) is a cardiodepressive agent and has negative effects on the heart. The study in dogs by Manders and Vatner reported that PB slightly affected arterial pressure and cardiac output, but markedly depressed left ventricular function and myocardial contractility [15]. Our data in animals without hemorrhagic shock are consistent with Manders and Vatner's work that PB decreased cardiac contractility and slightly altered cardiac out put. However, we found that PB progressively decreased MAP which is contradicted with their results. Yang et al. used an echocardiography to assess cardiac function in both conscious and anesthetized mice [26]. They reported that PB significantly altered cardiac hemodynamics and performance in mice, probably due to inhibition of sympathetic activity and myocardial contractility. Recently, the study about the effects of anesthetic agents on cardiac function in mice and rats using PV catheter has shown that PB caused the indices of systolic and diastolic function much lower than other anesthetics i.e. isoflurane and mixture of ketamine and xylazine [16]. However, our results suggest that PB majority impaired systolic and contractile function but slightly altered diastolic function in the hamster model. The depression in systolic function may be a result of suppression of plasma catecholamine (epinephrine and norepinephrine)

concentration by PB [2, 22]. Furthermore, PB inhibits the reflex activation of the sympathetic nervous system and causes hypothermia in animals [2, 12, 21-23]. PB also produces respiratory depression which can attenuate the gas exchange activity [3, 6, 7]. Conclusively, PB plays an important role in cardiac performance and compensatory responses.

Hemorrhagic shock progressively causes hypotension, hypothermia and reduction of oxygen delivery in consequence. These consequences significantly impair cardiac performance in the severe hemorrhagic shock. However, the degree of hemorrhagic shock depends on the amount of blood loss and the duration of shock [1, 13]. Using fixed volume moderate hemorrhagic shock in our study, dP/dt_{max} gradually decreased during the shock in the HS group, as same as that was observed in the SC group. This finding may imply that the effects of PB still dominated in the HS group. Other load-dependent systolic function index such as ejection fraction (EF) was higher than the baseline during the shock which was controversial with dP/dt_{max}. However, our results were in agreement with the work of Welte et al.[25]. Their study measured hemodynamic and cardiac contractility in anesthetized pigs under hypovolemic shock condition and showed that dP/dt_{max} decreased but EF increased at shock stage relative to baseline. There were several studies suggested that the cardiac contractility was increased during the shock as a result of autonomic response to the hypotension [11, 18]. Our results showed that other indices of systolic function such as P_{es}, $dP/dt_{max}/V_{ed}$ and approximated E_{es} apparently were increased during the shock period and higher than the baseline, implying the cardiac contractile function was enhanced due to the compensatory mechanism. However, there were some studies demonstrated that cardiac contractility

was depressed during hemorrhagic shock [1, 8, 9, 13, 14, 17]. Theoretically, myocardial contractility should be independent of preload, afterload and heart rate thus it is difficult to measure. Therefore, it is still controversial and the interpretation of cardiac contractility depends on the measurement techniques and animal models.

We also found that diastolic function of the heart was effectively depressed during the shock as demonstrated by the significant decrease in dP/dt_{min} and dV/dt_{max}. The relaxation time constant was significantly higher than baseline after hemorrhage, showing the depletion in the isovolumic relaxation phase. As the reduction of dV/dt_{max} during the shock, it indicated that the vascular return or preload was attenuated and the filling phase was delayed. When we considered the indices of diastolic function between the SC and HS groups, we found that the depression of diastolic function was strongly caused by the consequences of hemorrhage, not by the effects of PB.

The load dependent cardiac parameters such as SW and SV significantly decreased during the hemorrhagic shock. However, the work done per stroke volume (SW/SV) during the shock phase was maintained. This possibly indicates that the rate of energy consumption per unit volume was maintained even though hemorrhagic shock existed.

By this study, several concerns should be considered. First, the orientation and position of PV conductance catheter in the left ventricle can affect the accuracy of left ventricular volume. Second, the parallel volume may vary during hemorrhagic shock and play a role in the calculated parameters such as EF, E_{es} and $dP/dt_{max}/V_{ed}$. Third, the body core temperature of animals also influences the myocardial electrical conductivity and cardiac chronotropy.

In summary, our study showed that sodium pentobarbital and hemorrhage significantly affect on cardiac function in both systole and diastole. The roles of both sodium pentobarbital and hemorrhagic shock on the heart performance are presented as a diagram in Figure 2.10. Sodium pentobarbital inhibits sympathetic nervous system, leads to hypothermia, depresses respiratory system and attenuates cardiac performance. Hemorrhagic shock impaired oxygen delivery and released myocardial depressant factor. Therefore, when animals anesthetized with sodium pentobarbital were under moderate hemorrhagic shock, cardiac function was more depressive.

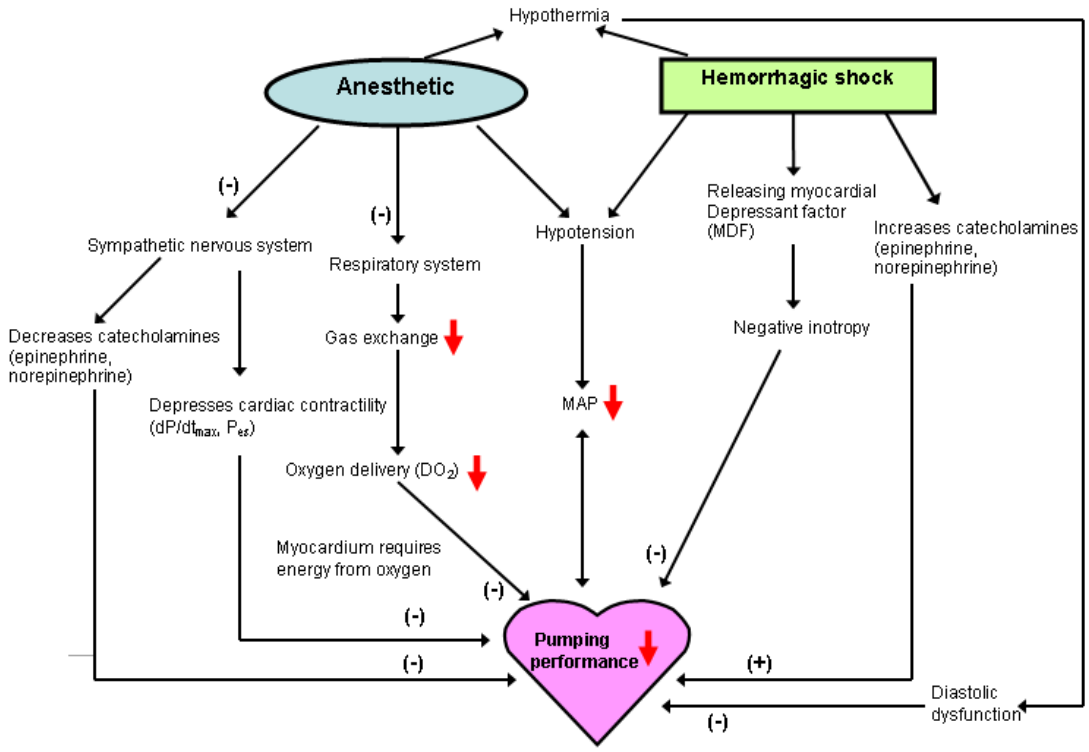

Figure 2.10: Schematic diagram for the roles of anesthetic and hemorrhagic shock on cardiac performance. Thick arrow represents a decrement. (+): positive effect and (-): negative effect

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Chapter 3

The Effects of Viscogenic Plasma Expanders on Cardiac Function: The Study in Moderate Hemorrhagic Shock-Resuscitation Model

Abstract

Plasma expanders (PEs) are used to restitute blood volume prior to blood transfusion. Elevating plasma viscosity with PEs, in awake animals, has shown the enhancement of blood circulation and the maintenance of tissue perfusion after hemorrhagic shock resuscitation. As microvascular perfusion improved after resuscitation with high viscosity PEs, it may imply a parallel improvement in cardiac function. Therefore, the viscosity of PEs may potentially influence the pumping performance of the heart, leading us to investigate the effect of PE viscosity on cardiac function after resuscitation in anesthetized hamster subjected to a 40% of blood volume hemorrhage. Resuscitation was performed by restitution of 20% of blood volume with LVPEs (polyethyletene glycol conjugated with human serum albumin (PEG-HSA), 2.2 cP and dextran 70 kDa, 2.9 cP) or HVPE (alginate, 7.2 cP). Cardiac function was assessed by means of a miniature conductance catheter to measure left ventricular pressure and volume. LVPEs significantly improved mean arterial blood pressure during 30 min after resuscitation relative to shock state while HVPE did not improve that. Cardiac output, stroke volume and stroke work, in animals resuscitated with LVPEs, markedly enhanced relative to shock state whereas these indices slightly increased in animals resuscitated with HVPE. Furthermore, LVPEs provided higher values of maximum rate of pressure change (dP/dt_{max}), minimum rate of pressure change (dP/dt_{min})

and maximum rate of volume change (dV/dt_{max}) compared with HVPE. All PEs caused a lower effective arterial elastance relative to shock state, indicating a reduction of afterload by volume restitution. In conclusion, increased plasma viscosity with alginate (HVPE), in an anesthetized hemorrhagic shock model, did not enhance cardiac performance over PEG-HSA and dextran 70kDa (LVPEs). However, alginate could sustain cardiac pumping performance after resuscitation, not markedly decline over time.

Keywords: hemorrhagic shock; resuscitation; plasma viscosity; cardiac function; conductance catheter

Introduction

Blood losses from the body during accidental injury or physical trauma causing multi-organ failure are critical issues in an emergency care unit. Hypovolemia and hypotension are the consequences after blood losses, leading to the reduction of the amount of RBCs which carry oxygen to tissues. This reduction lowers tissue oxygenation, leads to anaerobic metabolism, ultimately inducing multi-organ dysfunction. Blood or fluid infusions are used to treat volume deficit. Plasma expanders (PEs) are infused fluids which restitute blood volume by expanding the volume of plasma. Modulating plasma viscosity with PEs, in awake animals, shows the enhancement of blood circulation and the maintenance of tissue perfusion after hemorrhagic shock resuscitation [2, 19]. Systemic parameters showed significantly improved after hyperviscous PE infusion in a hemorrhagic shock resuscitation model compared to low viscosity PE infusion [3, 19].

Early study found that cardiac index and the capillary perfusion improved after volume resuscitation with high viscosity PE compared with low viscosity PE [19]. As blood perfusion improved after resuscitation with high viscosity PEs, it may imply a parallel improvement in cardiac function. Therefore, the viscosity of PEs may potentially influence the pumping performance of the heart.

Pumping performance of the heart can be assessed by many cardiac function indices such as cardiac contractility, cardiac output, stroke volume and stroke work. Few studies in anesthetized animals investigated the effects of infusion fluids such as hypertonic saline and dextran 70 on the cardiac function after shock resuscitation [9, 13, 14, 18]. Whereas, how viscosity of infused fluid affected cardiac function was not

considered in these studies. Therefore, for shock resuscitation, a role of PE viscosity on the cardiac function is still in question and is important to investigate.

The aim of this study was to examine the effect of PE viscosity on cardiac function after resuscitation in the moderate hemorrhagic shock model. In this study, anesthetized hamsters were subjected to a 40% of blood volume hemorrhage followed by 30 min of shock period. Resuscitation was performed by restitution of 20% of BV with dextran70 (Dx70), Poly Ethylene Glycol conjugated with human serum albumin (PEG-HSA) and alginate. PEG-HSA, Dx70 and alginate were used as low viscosity (LVPE), and high viscosity (HVPE) PEs, respectively. The results were also compared to hemorrhagic shock animals without resuscitation. To assess cardiac function, we applied a miniaturized conductance catheter to measure left ventricular pressure and volume. This technique provides a real time ventricular volume measurement and a benefit to simultaneously quantify load and interaction between heart and vasculature in each cardiac cycle.

Methods and Materials

Animal preparation

Studies were performed in anesthetized male Golden Syrian hamsters (Charles River Laboratories, Boston, MA) weighing 60-70g. Animal handling and care followed the NIH Guide for Care and Use of Laboratory Animals. The experimental protocol was approved by the local animal care committee. Animals were intraperitoneally administered with sodium pentobarbital (50mg/kg) in order to perform surgery and experiment. The left jugular vein catheterization was conducted to infuse fluids and the

left femoral artery was catheterized for blood pressure monitoring, blood withdrawal and blood sampling. Furthermore, animals were performed tracheal incision and cannulated with a polyethylene-90 tube to facilitate spontaneous breathing. Animals were put in the supine position on the heating pad to maintain the body temperature at 36-37^{o}C. During experiment, if animals response to a toe pinching, a small bolus of sodium pentobarbital (10-15mg/kg, i.p.) will be given.

Inclusion criteria

Animals were under anesthesia and were suitable for the experiments if: no bleeding and systemic parameters were within normal range, namely, mean blood arterial pressure (MAP) above 80 mmHg, heart rate (HR) above 320 beats/minute and systemic hematocrit (Hct) above 45%.

Systemic parameters

MAP and HR were monitored continuously (MP150, Biopac System Inc., Santa Barbara, CA), except when blood was sampled for laboratory parameters and blood conductance calibration and withdrawn for hemorrhage. The Hct was determined from centrifuged arterial blood samples taken in heparinized capillary tubes. Hemoglobin (Hb) content was measured by spectrophotometer (B-Hemoglobin, Hemocue, Stockholm, Sweden).

Blood chemistry and biophysical properties

Arterial blood was collected in a heparinized capillary tube (0.05ml) and immediately analyzed for arterial oxygen partial pressure (PaO_2), arterial carbon dioxide partial pressure ($PaCO_2$), base excess (BE) and pH (Blood Chemistry Analyzer 248, Bayer, Norwood, MA). Viscosity was measured at a shear rate $160s^{-1}$ (Brookfield

Engineering Laboratories, Middleboro, MA). Colloid osmotic pressure (COP) of PEs was measured using a membrane colloid osmometer (model 420, Wescon, Logan, UT).

Cardiac function

Closed chest method was performed in this study. Right common carotid artery was exposed for a 1.4F pressure-volume conductance catheter (PV catheter; SPR-839, Millar Instruments, TX) insertion and the PV catheter was advanced passing through the aortic valve into the left ventricle [15]. At the baseline, 15 minutes after hemorrhage and at the end of experiment, a bolus of 15% hypertonic saline (10 μl) was intravenously injected to determine the parallel volume (V_p). The pressure and volume signals were instantaneously digitized and acquired (MPVS300, Millar Instruments, TX and PowerLab 8/30, ADInstruments, CO).

Estimation of left ventricular blood volume

To determine the blood volume in the left ventricle, it is necessary to convert the measured blood conductance to the actual blood volume. The calibration of conductance catheter was performed using a series of 4 known-volume cylindrical cuvettes (14.14, 22.09, 31.81 and 43.30 μl). Blood volume measured by a conductance catheter was determined by $V_{lv} = S * RVU + C - V_p$; where V_{lv} is the absolute left ventricle volume, RVU is the blood conductance measured by PV catheter, S and C are the slope and the intercept of linear regression from blood calibration, respectively, as demonstrated in Figure 3.1 and V_p is the parallel volume caused by the tissues surrounding left ventricle.

Moderate hemorrhagic shock-resuscitation protocol

Anesthetized animals were withdrawn 40% of estimated blood volume (BV) via the femoral artery catheter within 15 minutes to induce moderate hemorrhage. Total BV

was estimated as 7% of body weight. The shock condition was hold for 30 minutes and then animals were resuscitated with 50% of the shed blood volume of study fluids (see Experimental group section) within 10 min via jugular vein catheter. The amount of resuscitated fluid was only 20% of blood volume which did not cause hypervolemia in animals. Systemic parameters (MAP, HR, Hb, Hct and blood chemistry) were monitored and analyzed at baseline, 30 min after shock and 60 min after resuscitation. The parameters were analyzed before hemorrhage (baseline), after hemorrhage (shock), and up to 60 min after volume replacement (resuscitation) as schematically shown in Figure 3.2.

Test solutions

High viscosity solution was prepared using 0.6% of LVM alginate (FMC Biopolymer, Brakøya, Norway) in 10% hydroxyethyl starch (HES). LVM alginate powder with a guluronic to mannuronic acid residue ratio of 30:70 was used. The 6% dextran 70 kDa in 0.9% NaCl (B. Braun Medical, Irvine, CA) was used as low viscosity solution. PEG-HSA was prepared using PEGylation conjugated with human serum albumin (HSA; Sigma-Aldrich, St. Louis, MO). The process for PEGylation and conjugation of PEG-HSA was previously described by Meng et al. [12] Table 3.1 shows the viscosity of the test solutions.

Experimental groups

Animals were randomly divided into the following groups: (1) Sham shock (SS) without fluid resuscitation, (2) PEG-HSA (PEG-HSA) resuscitated with 4g/dl PEG conjugated with human serum albumin, (3) Dextran 70 (Dx70) resuscitated with 6%

dextran 70 kDa in 0.9% NaCl and (4) Alginate (Alg) resuscitated with 0.6% alginate in 10% HES.

Data analysis

Cardiac function data were analyzed with PVAN software (version3.6, Millar Instruments, TX). Indices of systolic and diastolic function were calculated including maximum rate of pressure change (dP/dt_{max}), ejection fraction (EF), left ventricular end-systolic pressure (P_{es}), minimum rate of pressure change (dP/dt_{min}), ratio between dP/dt_{max} and end-diastolic volume ($dP/dt_{max}/V_{ed}$), maximum filling volume rate (dV/dt_{max}) and left ventricular end-diastolic pressure (P_{ed}). Other cardiac function indices such as cardiac out (CO), stroke work (SW) and stroke volume (SV) were also taken account. In addition, stroke work was normalized by stroke volume (SW/SV) representing the work done by a heart per unit volume. The values of studied cardiac function indices were averaged from selected 8-12 cardiac cycles at each time point.

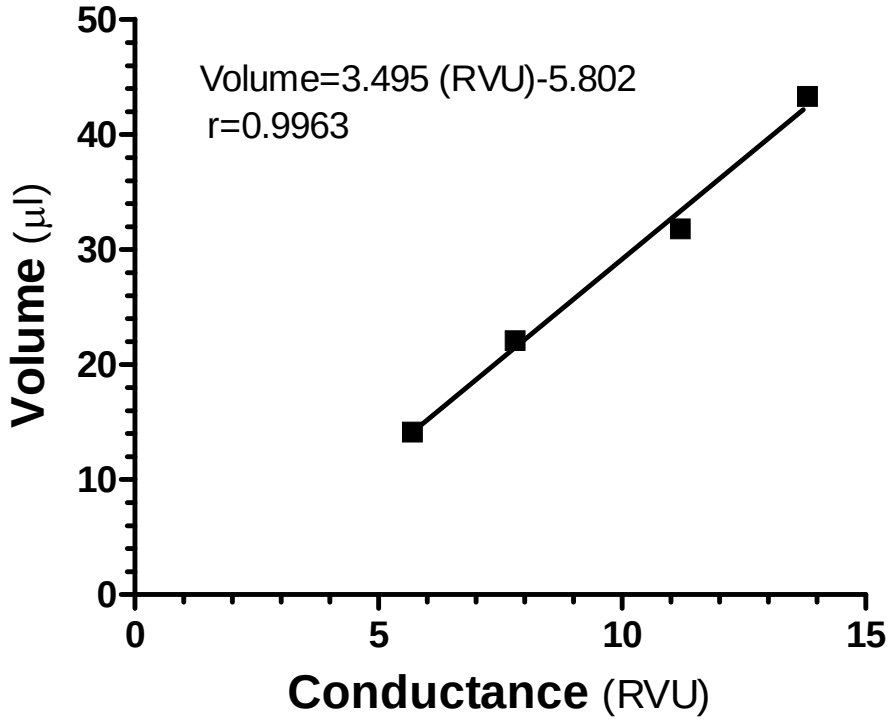

Figure 3.1: Linear regression of blood calibration from blood conductance (RVU) to real volume (µl) in each known-volume cylinder.

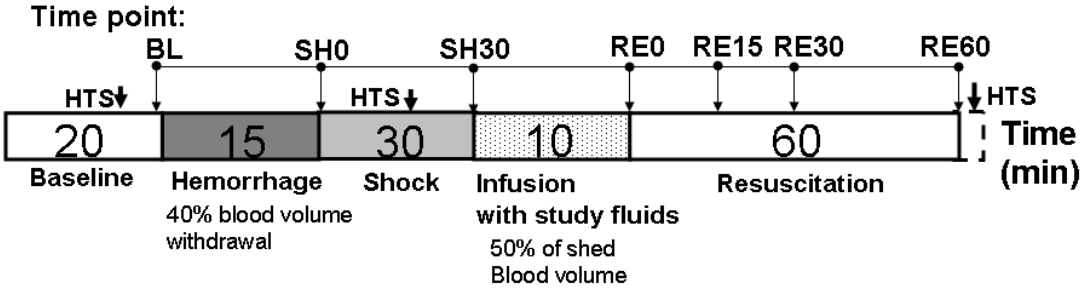

Figure 3.2: Schematic diagram of the fixed volume hemorrhagic shock-resuscitation protocol. BL, baseline; SH0, beginning of shock; SH30, 30 minutes after beginning of shock; RE0, beginning of monitoring; RE15, RE30, RE60; 15, 30, 60 minutes after end of resuscitation, respectively; HTS, hypertonic saline.

Table 3.1: Physical properties of the solutions

Solution	Viscosity (cP)	COP (mmHg)	Composition
PEG-HSA	2.2	58	4g/dl PEG+10% HSA
Dextran 70	2.9	52	6% dextran 70 kDa + 0.9% NaCl
Alginate	7.2	50	0.6% Alginate + 10% HES

Viscosity was measured at shear rate of 160 s^{-1} at 37^{o}C. Values are means.

Statistical analysis

Results are mean ± standard deviation (SD) unless otherwise noted. The values are mostly presented as relative to the level at 30min in shock (SH30). A ratio of 1.0 signifies no change from shock, whereas lower or higher ratios are indicative of changes proportionally lower or higher than the shock level. Data between interested time points in a same group were analyzed using analysis of variance for repeated measures (ANOVA) and followed by post hoc analyses with the Bonferroni's multiple comparison tests. Two-factor ANOVA test with a post hoc t-test with Bonferroni correction for multiple comparisons was used to compare between groups at time point of interest. All statistics were calculated using GraphPad Prism 4.01 (GraphPad Software, San Diego, CA). Results were considered statistically significant if $p<0.05$.

Results

Twenty animals were entered into the study and animals were randomly assigned to the following groups: SS (n=5; 66.2±2.7g), PEG-HSA (n=5; 66.2±2.6g), Dx70 (n=5; 67.4±1.7g) and Alg (n=5; 67.4±2.4g).

Systemic parameters

Table 3.2 presents systemic and blood gas parameters measured at baseline, 30min after hemorrhage (SH30) and 60min after resuscitation (RE60) for all study groups. For systemic and blood gas parameters, no significant differences between groups were found at the baseline and 30 min after hemorrhage (SH30). As expected, animals in the SS group had significant decreases of blood gas parameters after hemorrhage relative to baseline. Animals in the group resuscitated with PEG-HSA showed the lowest PaO_2 after

resuscitation. The arterial pH was elevated in the groups resuscitated with PEG-HSA and Dx70 compared with the SS group ($p<0.05$). The base excess in the resuscitated groups was significantly less negative compared with the SS group ($p<0.05$). The groups resuscitated with the test solutions showed a significant reduction of Hct after resuscitation compared with the baseline, SH30 and the SS group ($p<0.05$). The hemoglobin content also showed a similar trend as Hct.

Animals in the SS group showed a continuous drop in MAP over the observation time. PEG-HSA and Dx70 significantly improved MAP during 30 min after resuscitation relative to SH30 while alginate did not improve that. There was a significant difference in MAP between groups comparing with the SS group when animals resuscitated with PEG-HSA, Dx70 and alginate (Figure 3.3; $p<0.05$). At 60 min after resuscitation, only animals in the PEG-HSA group were significantly observed a higher MAP relative to animals in the SS group ($p<0.05$). However, all groups showed a tentative decrease of MAP over the time after 15 min post-resuscitation.

Pressure-volume loop

Figure 3.4 demonstrated the examples of pressure-volume loop (PV loop) at three time points in this study. Hemorrhage shifted PV loop to the left of the PV loop at the baseline. PV loop during the shock was also smaller relative to that at the baseline. After resuscitation, PV loop was shifted toward to PV loop at the baseline and became bigger compared with PV loop at the shock phase.

Left ventricular systolic indices

The dP/dt_{max} in the SS group gradually decreased over the observation time and significantly dropped at the late stage of the experiment (RE30 and RE60) relative to

SH30 (Figure 3.5A; $p<0.05$). On the other hand, dP/dt_{max} in the PEG-HSA and Dx70 groups after resuscitation apparently increased and was higher than that at the SH30. At the early stage after resuscitation, Dx70 increased dP/dt_{max} significantly compared with the SS group to about 40% ($p<0.05$). In both the PEG-HSA group and the Alg group, no statistical significance in dP/dt_{max} was found after resuscitation relative to SH30. Furthermore, after resuscitation, dP/dt_{max} in the Alg group showed a continuous decrease and was lower than the value at the shock.

The EF of four groups after resuscitation was unchanged compared with SH30 as presented in Figure 3.5B. There was also no significant change of EF among groups over the time after resuscitation.

The P_{es} in the SS group gradually decreased while it was unchanged in the PEG-HSA and Alg groups after resuscitation as shown in Figure 3.5C. In the Dx70 group, P_{es} at 15 min after resuscitation was higher than that at SH30 and then P_{es} gradually dropped over the entire remaining period. Whereas, P_{es} in the PEG-HSA and Alg groups was maintained lower than SH30. No significant change in P_{es} was found either within group or between groups.

Left ventricular diastolic indices

The dP/dt_{min} after resuscitation in the SS and Alg groups was slightly lower than at the shock phase as demonstrated in Figure 3.6A ($p<0.05$). In contrast, dP/dt_{min} after resuscitation in the Dx70 group was 20-50% higher than SH30 and significantly increased at the early stage after resuscitation (RE15; $p<0.05$). There was also a significant enhancement of dP/dt_{min} after resuscitation with dextran70 compared with resuscitation with alginate and without resuscitation ($p<0.05$). Animals resuscitated with

PEG-HSA had an improvement in dP/dt_{min} during 30 min after resuscitation relative to at the shock phase.

The dV/dt_{max} in all resuscitated groups, after resuscitation, was higher than the values at the SH30 as shown in Figure 3.6B. There was a significant improvement of dV/dt_{max} in the PEG-HSA group after resuscitation compared with SH30 ($p<0.05$). No change of dV/dt_{max} relative to SH30 was found in the SS group.

The P_{ed} in the SS group did not significantly change over the time relative to SH30 as presented in Figure 3.6C. In contrast, P_{ed} after resuscitation in the Dx70 group showed a significant increase compared with the values at the SH30 ($p<0.05$). However, resuscitation with alginate and PEG-HSA did not significantly improve P_{ed} compared with at the shock phase.

Table 3.2: Laboratory parameters

	Group	MAP (mmHg)	HR (bpm)	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	Arterial pH	BE (mmol/l)	Hct (%)	Hb (g/dl)
Baseline (BL)	SS	84 (9)	491 (28)	48.5 (4.9)	45.5 (8.6)	7.34 (0.07)	-1.6 (3.7)	49 (3)	14.7 (1.3)
	PEG-HSA	91 (6)	466 (25)	39.4 (4.6)	44.7 (6.0)	7.35 (0.04)	-1.3 (5.3)	47 (2)	14.6 (0.6)
	DX70	85 (3)	450 (52)	43.1 (9.8)	52.6 (6.1)	7.34 (0.03)	0.7 (2.3)	50 (3)	16.1 (0.8)
	Alg	90 (9)	447 (20)	43.4 (9.8)	46.2 (10.3)	7.33 (0.07)	-2.5 (2.5)	51 (2)	15.2 (1.0)
30 min Shock (SH30)	SS	56 (8)***	453 (32)*	71.6 (10.5) **	38.0 (5.1)	7.21 (0.07)	-12.1 (4.5) *	33 (2) ***	9.7 (0.3) ***
	PEG-HSA	60 (5)***	461 (19)	66.6 (28.0)	42.1 (12.1)	7.32 (0.14)	-8.2 (2.6)	36 (3) ***	11.0 (1.1) ***
	DX70	53 (8)***	435 (38)	87.56 (21.9) **	33.8 (6.9) ***	7.25 (0.13)	-11.8 (3.9) **	37 (3) ***	11.1 (1.4) ***
	Alg	60 (4)***	455 (35)	65.92 (31.0)	38.2 (11.8)	7.26 (0.10)	-9.9 (2.7)	37 (2) ***	11.0 (0.8) ***
60 min Resuscitation (RE60)	SS	41 (4)***	425 (33) ***	87.3 (20.3) ***,##	32.9 (4.5)	7.11 (0.11) *	-17.9 (4.1) **	30 (4) ***,#	9.1 (0.8) ***
	PEG-HSA	62 (8)***,†††	458 (25)	49.9 (9.3)%	43.2 (1.9)	7.35 (0.07) ††	-2.1 (4.0) †††	24 (3) ***,###,†††	7.5 (1.0) ***,###,†
	DX70	53 (8)***,#	445 (24)	80.0 (25.3) **	32.5 (6.1) ***	7.33 (0.07) †	-8.1 (4.4) *, ††	22 (3)***,###,†††	7.3 (1.0) ***,###,†
	Alg	51 (14)***	478 (37)	70.1 (33.8)	37.3 (16.0)	7.27 (0.22)	-10.1 (7.5) †, ‡	22 (4) ***,###,†††	7.5 (0.9) ***,###,†

Values are means (SD). MAP, mean arterial pressure; HR, heart rate; PaO_2, arterial partial O_2 pressure; $PaCO_2$, arterial partial CO_2 pressure; BE, base excess; Hct, systemic hematocrit; Hb, hemoglobin content of blood. * p<0.05; ** p<0.01; *** p<0.001 compared with baseline. # p<0.05; ### p<0.001 compared with SH30. † p<0.05; †† p<0.01; ††† p<0.001 compared with SS group. ‡ p<0.05 compared with PEG-HSA group.

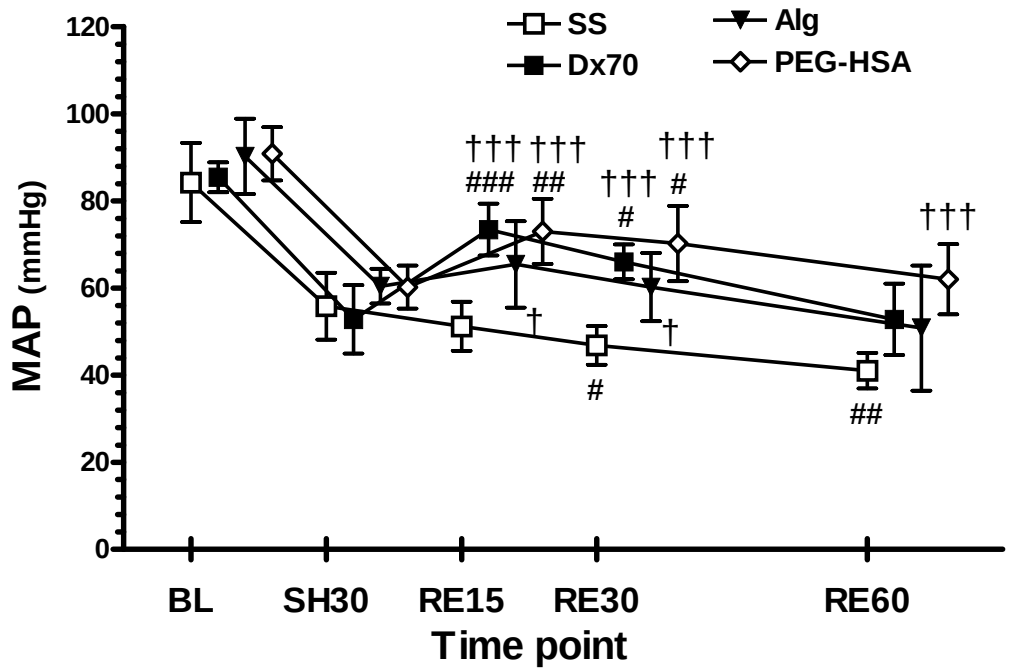

Figure 3.3: Mean arterial pressure (MAP) measured at baseline (BL), at 30min after hemorrhage (SH30), at 15, 30 and 60 minutes after resuscitation (RE15, RE30, RE90). Values are presented as means ± SD. # $p<0.05$; ## $p<0.01$; ### $p<0.001$ compared to SH30. † $p<0.05$; ††† $p<0.001$ compared with SS group.

Cardiac function parameters

Animals resuscitated with PEG-HSA and Dx70 showed 40-50% and 30-40% improvement of CO relative to SH30 and compared with the SS group, respectively (Figure 3.7A; $p<0.05$). After resuscitation, CO in the Alg group was higher than the value at the SH30 but it was not statistically significant.

The SW after resuscitation with Dx70 was significantly increased at the early stage after resuscitation (RE15 and RE30) relative to SH30 while animals resuscitated with PEG-HSA significantly showed an enhanced SW for the entire period after resuscitation (Figure 3.7B; $p<0.05$). Furthermore, significant differences of SW were found between the PEG-HSA group and the Alg group at 15 min, 30 min and 60 min after resuscitation ($p<0.05$). Animals resuscitated with PEG-HSA and Dx70 had SW about 50-65% and 25-50% higher than animals without resuscitation, respectively ($p<0.05$).

The SV after resuscitation with PEG-HSA significantly increased 40-55% relative to SH30 while resuscitation with Dx70 increased SV 30-35% relative to SH30 (Figure 3.7C; $p<0.05$). Resuscitation with alginate also increased SV greater than the value at SH30 but it was not statistically significant. In addition, only statistical significance in SV between the PEG-HSA group and the SS group was found ($p<0.05$).

A significant reduction of the SW/SV ratio was observed at RE30 and RE60 in the SS group (Figure 3.7D; $p<0.05$). At 15 min after resuscitation with dextran 70, the SW/SV ratio was significantly elevated ($p<0.05$). Resuscitation with dextran 70 also showed a higher significance of this ratio than resuscitation with alginate at 15 min after

resuscitation (p<0.05). The SW/SV ratio was maintained after resuscitation with alginate and it was lower than the value at the SH30.

Effective arterial elastance

The effective arterial elastance (Ea) calculated by end-systolic pressure normalized by stroke volume showed a significant reduction after resuscitation with PEG-HSA and alginate compared with the value at SH30 as presented in Figure 3.8A (p<0.05). The Ea in the Dx70 group was statistically significant only at 60 min after resuscitation relative to SH30 (p<0.05).

Systemic vascular resistance

Resuscitation with PEs caused a lower systemic vascular resistance (SVR) compared with shock phase. Only animals in the PEG-HSA group had a significant reduction of SVR relative to SH30 (p<0.05) while other groups were not observed.

Viscosity of blood and plasma

Table 3.3 compares viscosities of blood and plasma at 60 min after resuscitation. Blood viscosity was reduced about 50% of the baseline and plasma viscosity was maintained as similar as normal blood plasma after resuscitation with dextran70 or PEG-HSA. Alginate reduced blood viscosity to 75% of the baseline and increased plasma viscosity about 2 times from the baseline.

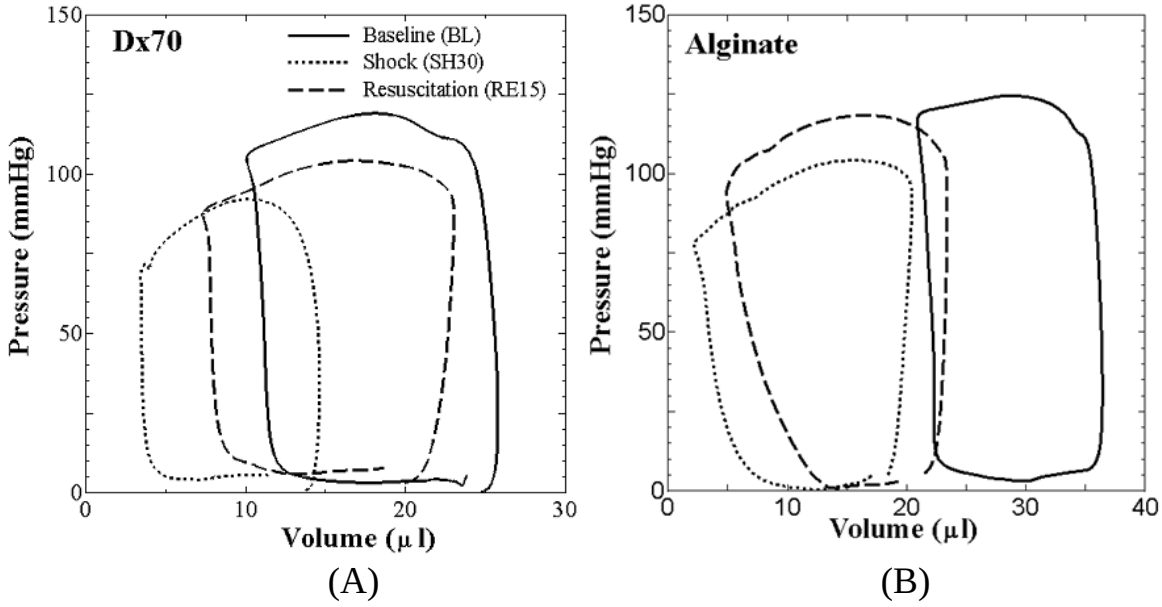

Figure 3.4: Left ventricular pressure-volume loops (PV loops) derived by PV conductance catheter. PV loops at baseline, 30 min in shock and after resuscitation with (A) dextran70 kDa (Dx70) and (B) Alginate.

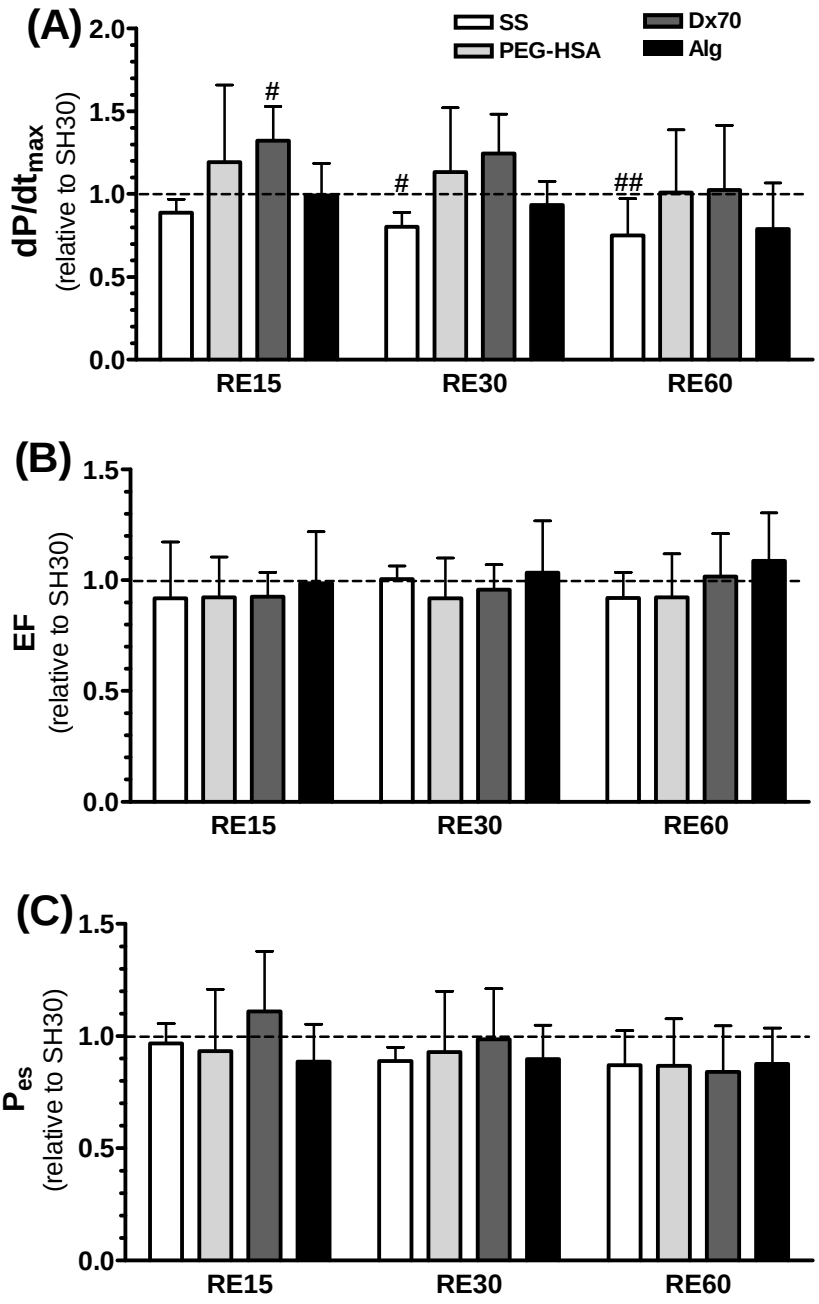

Figure 3.5: Left ventricular systolic function indices derived by PV conductance catheter. (A) Maximum rate of pressure change (dP/dt_{max}) after resuscitation. (B) Ejection fraction (EF) after resuscitation. (C) Left ventricular end-systolic pressure (P_{es}) after resuscitation. Broken line represent at 30min shock level. Values are presented as means ± SD. # $p<0.05$; ## $p<0.01$; ### $p<0.001$ compared with 30min shock. Time points as in Figure 3.3.

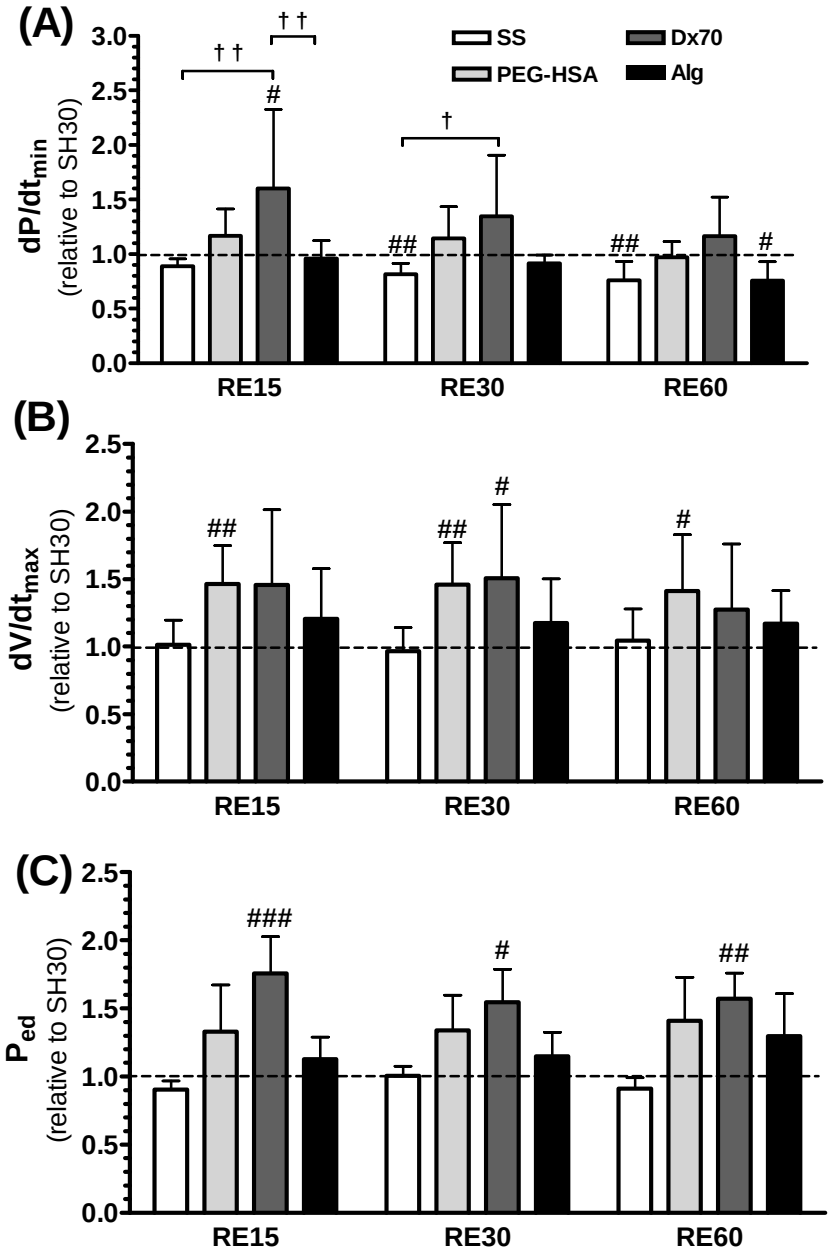

Figure 3.6: Left ventricular diastolic function indices derived by PV conductance catheter. (A) Minimum rate of pressure change (dP/dt_{min}) after resuscitation. (B) Maximum filling volume rate (dV/dt_{max}) after resuscitation. (C) Left ventricular end-diastolic pressure (P_{ed}) after resuscitation. Broken line represent at 30min shock level. Values are presented as means ± SD. # p<0.05; ## p<0.01; ### p<0.001 compared with 30min shock. † p<0.05; †† p<0.01; between groups. Time points as in Figure 3.3.

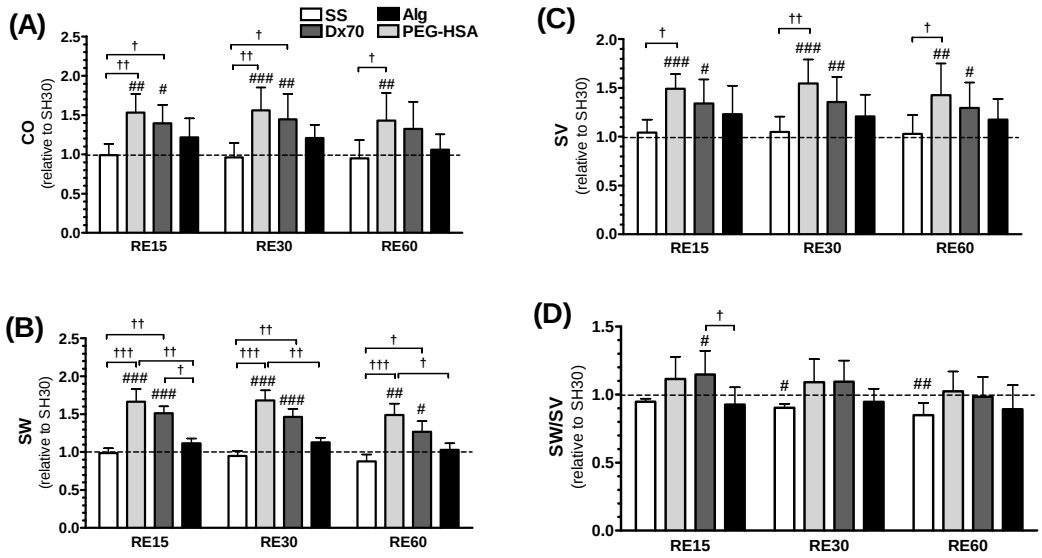

Figure 3.7: Other left ventricular cardiac function indices derived by PV conductance catheter. (A) Cardiac output (CO) after resuscitation. (B) Stroke work (SW) after resuscitation. (C) Stroke volume (SV) after resuscitation. (D) Work done per stroke volume (SW/SV) after resuscitation. Broken line represent at 30min shock level. Values are presented as means ± SD. # $p<0.05$; ## $p<0.01$; ### $p<0.001$ compared with 30min shock. † $p<0.05$; †† $p<0.01$; ††† $p<0.001$ between groups. Time points as in Figure 3.3.

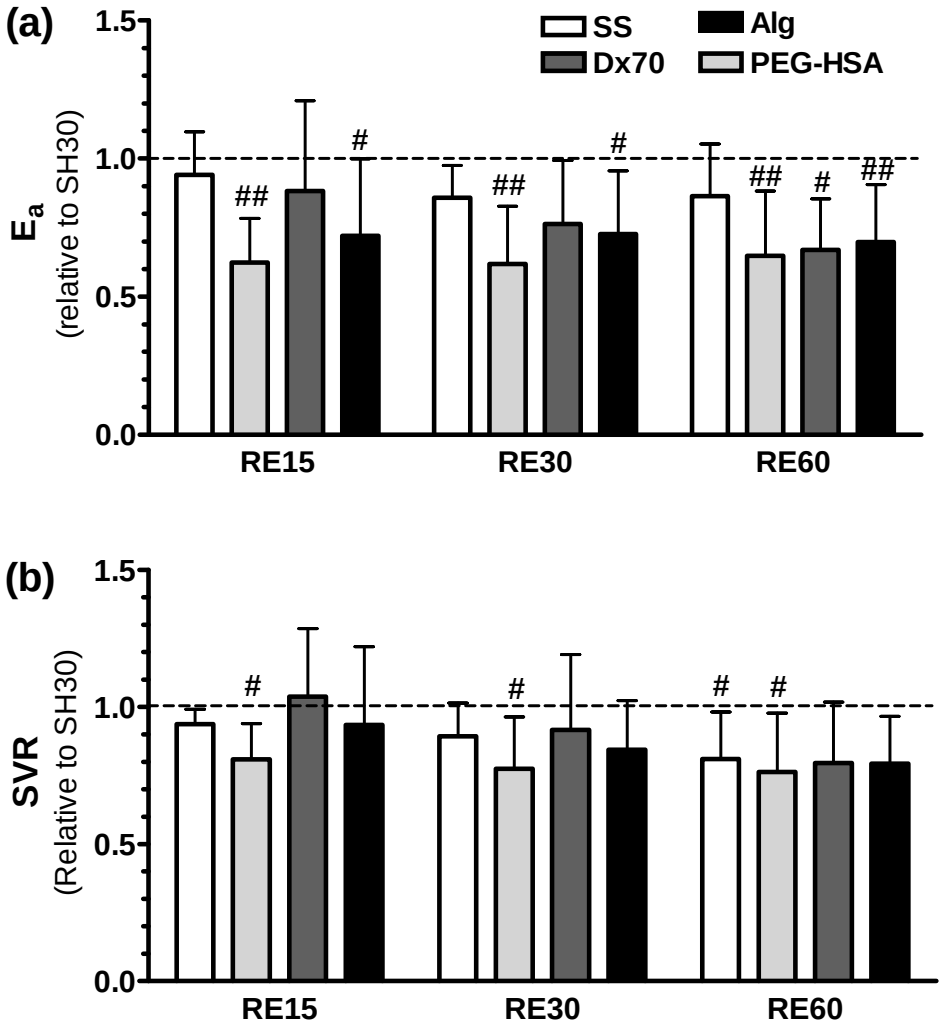

Figure 3.8: (A) Effective arterial elastance (E_a) derived by PV conductance catheter. (B) Systemic vascular resistance (SVR) calculated from mean arterial pressure and cardiac output. Broken line represents at 30min shock level. Values are presented as means ± SD. # p<0.05; ## p<0.01 compared with 30min shock. Time points as in Figure 3.3.

Table 3.3: Blood and plasma viscosity at 60 min after resuscitation

60 min after resuscitation	**Hct (%)**	**Blood viscosity (cP)**	**Plasma viscosity (cP)**
Blood (Baseline)	51	4.8	1.1
Blood (Shock)	30	2.7	1.1
PEG-HSA	24	2.4	1.1
6% Dextran 70	22	2.5	1.3
0.6% Alginate	22	3.6	2.3

Viscosity was measured at shear rate of 160 s^{-1} at 37^{o}C. Values are means.

Discussions

The present study assessed cardiac function after resuscitation with three different viscogenic plasma expanders; polyethylene glycol conjugated with human serum albumin (PEG-HSA), dextran 70 kDa (Dx70) and alginate. Our main finding demonstrated that resuscitation with PEG-HSA and Dx70, LVPEs, elucidated comparatively better cardiac function compared with resuscitation with alginate, HVPE. PEG-HSA and Dx70 enhanced the indices of systolic and diastolic function such as dP/dt_{max}, dP/dt_{min}, CO and SW more effective than alginate. However, all of PEs relatively improved cardiac function after resuscitation especially diastolic function and volume-related parameters.

The improved systemic parameters and diastolic and systolic function after resuscitation were the results of volume restitution with PEs. PEG-HSA, Dx70 and alginate recovered the intravascular fluid and increased the venous return in comparison with shock phase as we could observe in the increases of SV and CO. This is also evidenced by the observed increase of work done that the heart produced to pump out blood. The increase of intravascular fluid is a result of colloid osmotic pressure that mobilizes fluid from the extravascular space into the intravascular space. The studies in awake animals with hemorrhagic shock and resuscitation model reported that fluid resuscitation increased cardiac output and arterial blood flow and improved microcirculation blood perfusion [5, 8, 19]. Their findings are in agreement with and support our findings in anesthetized hamsters.

All studied PEs showed a similar result in reduction of E_a, indicating that PEs lower afterload. Generally, the lower afterload implies the lower aortic pressure that the heart must eject blood against. This finding also implies that all studied PEs reduce the

systemic vascular resistance (SVR) immediately after resuscitation (10-15 min). Early studies in microcirculation elucidated that increased plasma viscosity decreased SVR as a result of vasodilation due to increased vascular wall shear stress [4-6, 11]. Additionally, increased plasma viscosity restored shear stress in microcirculation after resuscitation. Shear stress enhanced the rate of production of vasodilators (nitric oxide and prostacyclin) via endothelial mechanotransduction [6, 7, 10, 20]. Shear stress is a function of fluid viscosity and shear rate. In our study, we found that cardiac output after resuscitation with PEG-HSA and Dx70 was significantly higher than after resuscitation with alginate, indicating higher blood flow and shear rate were obtained. Although alginate provided blood and plasma viscosity higher than PEG-HSA and Dx70 did, alginate caused the lower cardiac output compared with others. Therefore, alginate possibly did not increase higher shear stress compared with other PEs in our study, leading a non-effective result of high viscosity PE.

Lower blood flow and blood pressure signify lower blood perfusion. As lower cardiac output in animals resuscitated with alginate, blood perfusion in the heart is possibly lower when resuscitation with alginate than PEG-HSA and Dx70. This result suggested that cardiac performance in the animals resuscitated with alginate is less efficient than that in the animals resuscitated with PEG-HSA or Dx70.

Our study demonstrated that PEG-HSA and Dx70 improved and maintained the load dependent cardiac function indices i.e. dP/dt_{max}, dP/dt_{min}, SW, SV, CO and dV/dt_{max} better than alginate. These results suggest that, in anesthetized animals, resuscitation with LVPE provided more beneficial effects than resuscitation with high viscosity PE. In contrast, early studies in awake animals showed that increased plasma viscosity after

volume replacement prolonged resuscitation and improved blood pressure and microvascular circulation in a shock model [2-4, 11].

The heart consumes energy converted from oxygen to eject blood. If we consider the heart like a pump, the heart should consume more energy to pump out high viscosity fluid than low viscosity fluid. Whereas, our study observed that stroke work (SW) or the work done by the heart per unit volume (SW/SV ratio) were lower in animals resuscitated with alginate than that in animals resuscitated with LVPEs; PEG-HSA and Dx70. This finding indicates an insufficient energy transfer to blood when resuscitation with alginate. In addition, two animals died during resuscitation with alginate and these animals were excluded in our analysis. We derived the mathematical relationship between SW and fluid viscosity from the simplified PV loop as described in the Appendix I. We found that stroke work (SW) was inversely proportional to fluid viscosity (μ); suggesting high viscosity reduces the external work produced by the heart which is different from an intuitive concept. However, this relationship also includes other parameters that can affect the stroke work such as heart rate (HR), vascular diameter (r), ventricular pressure (P_{ed} and P_{es}) and systemic mean arterial pressure (MAP).

As our study was performed in anesthetized shock animals, the effects of sodium pentobarbital and shock condition effectively attenuated the oxygen delivery and gas exchange. These attenuations may strongly affect on the oxygen- energy conversion of the heart when resuscitation with HVPE. Furthermore, our previous study showed that sodium pentobarbital also depressed the contractile function and relaxation function of the heart. Sodium pentobarbital also inhibits the sympathetic nervous activities that impact on the compensatory mechanisms and causes animals to be less tolerance to

hemorrhagic shock [1, 16, 17, 21]. Therefore, increased plasma viscosity using the sodium pentobarbital-anesthetized shock model demonstrated the different findings compared with the findings in awake animals with a shock model.

In summary, increased plasma viscosity with alginate (HVPE), in an anesthetized hemorrhagic shock model, did not enhance cardiac performance over PEG-HSA and Dx70 (LVPEs). This non-beneficial effect of alginate may be the results of the lower cardiac out put, the insufficient energy to eject blood and the inhibitory effects of sodium pentobarbital on sympathetic nervous system. Although viscosity of PEG-HSA was lower than viscosity of Dx70, PEG-HSA provided similar effects on heart performance as Dx70 did. It is possible that PEG-HSA might have other mechanisms or interactions with vascular system that produced the similar beneficial effect as providing by an increased plasma viscosity with Dx70. However, our study performed only three values of viscosity (2.2, 2.9 and 7.2 cP). It can not completely conclude that increase of plasma viscosity has no beneficial effect in an anesthetized hemorrhagic shock-resuscitation model. Further investigations are necessary to find out which range of PE viscosity provides the beneficial effect on cardiac function in an anesthetized hemorrhagic shock-resuscitation model.

Acknowledgements

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cardiac function after hemorrhagic shock and sustains resuscitation" by Chatpun S., Tsai A. and Intaglietta M. in 2009 IEEE International Symposium on Biomedical Engineering, Thailand, December 2009. Copyright by the Institute of Electrical and Electronics Engineers (IEEE). The dissertation author was the primary investigator and author of this paper.

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Chapter 4

The Effects of Viscogenic Plasma Expanders on Cardiac Function: The Study in Acute Isovolemic Hemodilution Model

Abstract

Previous studies had found that increasing plasma viscosity as whole blood viscosity decrease has beneficial effects in microvascular hemodynamics. As the heart couples with systemic vascular network, changes in plasma and blood viscosity during hemodilution determine vascular pressure drop and flow rate, which influence cardiac function. The purpose of this study was to investigate how plasma viscosity affects on cardiac performance during acute isovolemic hemodilution. Plasma viscosity was modulated by hemodilution with three different viscogenic plasma expanders (PEs). Dextran 2000 kDa (Dx2M, 6.4 cP) and dextran 70 kDa (Dx70, 3.0 cP) were used as high and low viscogenic PEs, respectively. Polyethylene glycol conjugated with human serum albumin (PEG-HSA, 2.2 cP) was also used as low viscogenic PE. Anesthetized hamsters were hemodiluted by 40% of estimated blood volume with plasma expanders. The cardiac function indices were measured using a miniaturized pressure-volume conductance catheter. Hemodilution with PEG-HSA, Dx70 and Dx2M increased plasma viscosity by 9%, 18% and 90% from baseline, respectively. Mean arterial pressure, at 60 min after hemodilution, dropped to 84%, 79% and 78% of the baseline in a group hemodiluted with Dx2M, Dx70 and PEG-HSA, respectively ($p<0.01$). End-systolic pressure and minimum rate of pressure change (dP/dt_{min}) significantly decreased relative to baseline in all groups ($p<0.05$). Furthermore, stroke volume markedly enhanced

compared to baseline after hemodilution in all groups while cardiac output significantly increased only in a group hemodiluted with Dx2M and PEG-HSA. Dx2M significantly caused higher stroke work relative to baseline and compared to Dx70 at 60 min after hemodilution ($p<0.05$). All PEs promisingly reduced systemic vascular resistance during hemodilution. In summary, our results demonstrated that PEG-HSA, in an acute hemodilution model, provided beneficial effects on cardiac function as similar as Dx2M and better than Dx70 although its viscosity was markedly lower than viscosity of Dx2M and Dx70. This suggests that PEG-HSA possibly has an interaction mechanism with vasculature different from high viscogenic plasma expander that is not related to viscosity.

Keywords: hemodilution; plasma expander; plasma viscosity; cardiac function; conductance catheter

Introduction

Plasma expanders (PEs) are intravenously infused fluids using for blood volume replacement therapy and preoperative blood management. The principal issues of PEs are fluid volume, intravascular circulating time, coagulation-related variables, safety and cost. Crystalloid and colloid solutions are commonly used PEs. Early studies have shown that crystalloids lead to tissue edema, causing respiratory distress syndrome [8, 12, 18, 20]. Colloids provide lasting circulating volume expansion and hemodynamic stability due to their oncotic properties [8, 12, 19, 23]. However, use of colloids for volume replacement in critically ill patients also affects on mortality and is more expensive than crystalloids [1, 24]. Therefore, there are still controversial issues for the advantages of crystalloids and colloids as administered fluids.

Recently, it has been proposed that the conjugation of protein with polyethylene glycol (PEG) such as PEG-conjugated human serum albumin (PEG-HSA) and hemoglobin (PEG-Hb) may be the next step of PEs [7, 26-28]. The conjugation of protein with PEG provides longer circulating time, lowers the toxicity and immunologic reactions and increases solubility in water. Recent animal studies suggest that this new type of molecules potentially has characteristics as PE [3, 7, 14, 17]. Cabrales et al. performed an extreme hemodilution with PEG-Hb and PEG-HSA in an awake hamster window model and found that these PEG compounds maintained microvascular conditions with lower concentrations than conventional PEs such as dextran 70 kDa (Dx70) [7]. These results were similar when using high viscogenic PEs, alginate and dextran 500 kDa, compared with Dx70 in an extreme hemodilution model [4]. Martini and colleagues also showed that 50% blood volume transfusion exchange with PEG-HSA

to stimulate preoperative hemodilution followed by 60% blood volume hemorrhage prolonged the maintenance of microvascular function better than the exchange with a conventional hydroxyethyl starch (HES) [14]. Therefore, it seems that PEG compounds similarly affect on microvascular function as high viscogenic PEs perform. Although many microcirculation studies have been performed, there still have many interesting key issues for PEG compounds such as the effects of PEG compounds on cardiac function and organ blood flow distribution.

The principal aim of this study was to evaluate the effect of PEG-HSA (viscosity 2.2 cP) on cardiac and vascular function compared to higher viscosity PEs, 6% dextran 2000 kDa (viscosity 6.3 cP) and 6% dextran 70 kDa (viscosity 3.0 cP) by using an acute isovolemic hemodilution (28% Hct) in anesthetized hamsters. Moderate isovolemic hemodilution was performed by a 40% blood volume exchange with test PEs followed by 1 hr monitoring period. To accomplish this goal, we evaluated left ventricular function indices derived from the pressure-volume (PV) measurement using a miniaturized PV conductance catheter. This technique provides a real time ventricular volume measurement and a benefit to simultaneously quantify load and interaction between heart and vasculature in each cardiac cycle.

Methods and Materials

Animal preparation

Studies were performed in anesthetized male Golden Syrian hamsters (Charles River Laboratories; Boston, MA) weighing 60-70g. Animal handling and care followed the NIH Guide for Care and Use of Laboratory Animals. The experimental protocol was

approved by the local animal care committee. Surgery was performed following i.p. injection of sodium pentobarbital (50mg/kg). The left jugular vein was catheterized to allow fluid infusion and left femoral artery was cannulated for blood pressure monitoring and blood withdrawal and sampling. Furthermore, Tracheotomy was performed and cannulated with a polyethylene-90 tube to facilitate spontaneous breathing. Animals were placed in the supine position on the heating pad to maintain the body temperature at 36-37^{o}C. During experiment, if animals response to a toe pinching, a small bolus of sodium pentobarbital (10-15mg/kg, i.p.) will be given.

Inclusion criteria

Animals under anesthesia were suitable for the experiments if : no bleeding and systemic parameters were within normal range, namely, mean blood arterial pressure (MAP) above 80 mmHg, heart rate (HR) above 320 beats/minute and systemic hematocrit (Hct) above 45%.

Systemic parameters

The MAP was monitored continuously (MP150, Biopac System Inc.; Santa Barbara, CA), except during blood exchange. The Hct was determined from centrifuged arterial blood samples taken in heparinized capillary tubes.

Biophysical properties

Viscosity was measured at a shear rate 160s^{-1} (Brookfield Engineering Laboratories, Middleboro, MA). Colloid osmotic pressure (COP) of PEs and blood plasma was determined using a membrane colloid osmometer (model 420, Wescor, Logan, UT).

Cardiac function

Closed chest method was performed to assess cardiac function in this study [21]. The right common carotid artery was exposed allowing a 1.4F pressure-volume conductance catheter (PV catheter; SPR-839, Millar Instruments, TX) to be inserted. The PV catheter was advanced passing through the aortic valve into the left ventricle. At the baseline and the end of experiment, a bolus of 15% hypertonic saline (10 μl) was intravenously injected to determine the parallel volume (V_p) [2]. The pressure and volume signals were instantaneously digitized and acquired (MPVS300, Millar Instruments, Houston, TX and PowerLab 8/30, ADInstruments, Colorado Springs, CO).

Moderate isovolemic hemodilution protocol

Anesthetized animals were exchanged by 40% of estimated blood volume (BV) with the test solution, lowering systemic Hct by 45%. Total BV was estimated as 7% of body weight. Test solutions were infused into the left jugular vein catheter at a rate of 100μl/min with simultaneous blood withdrawal at the same rate from the left femoral artery catheter by using a dual syringe pump (33 syringe pump, Harvard Apparatus, Holliston, MA). Blood samples were collected at the end of experiment for subsequent measurement of viscosity, plasma colloid osmotic pressure and blood conductance. Animals were monitored for 60min after complete the hemodilution. Systemic parameters (MAP and Hct) were recorded and analyzed at baseline and 60 min after hemodilution. Figure 4.1 illustrates the experimental protocol.

Test solutions

Three solutions were as follows: 1) 6% dextran T2000 (Pharmacosmos, Holbaek, Denmark) in 0.9% sodium chloride (NaCl) mixed with 10% human serum albumin , 2)

6% dextran 70 in 0.9% NaCl (B. Braun Medical, Irvine, CA) ,and 3) PEG-HSA was prepared by PEGylation conjugated with human serum albumin (HSA; Sigma-Aldrich, St. Louis, MO). The process for PEGylation and conjugation of PEG-HSA was previously described by Meng et al. [16] Table 4.1 lists the physical properties of these test solutions.

Experimental groups

Animals were randomly divided into three groups: 1) 6% dextran 2000 kDa (group labeled Dx2M), 2) 6% dextran 70 kDa (group labeled Dx70), and 3) PEG conjugated with HSA (group labeled PEG-HSA).

Estimation of left ventricular blood volume

Left ventricular blood volume was measured continuously in conductance units (RVU; relative volume unit) and converted to actual blood volume (μl) at the end of the experiment. (see Appendix II)

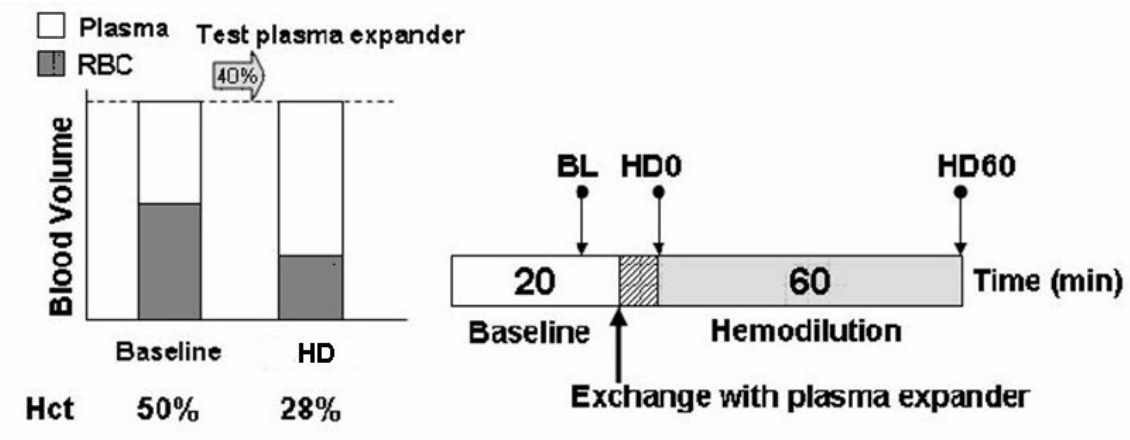

Figure 4.1: Schematic diagram of an acute isovolemic hemodilution protocol. Baseline characterization was performed before hemodilution. Blood volume was exchanged for 40% of blood volume with test plasma expanders. Observation period was 60 minutes after exchange. Hct, hematocrit; BL, baseline; HD, hemodilution.

Table 4.1: Physical properties of the solutions

Solution	Viscosity (cP)	COP (mmHg)
PEG-HSA	2.2	58
Dx70	3.0	52
Dx2M	6.3	43

Viscosity was measured at shear rate of 160 s^{-1} at 37^{o}C.
Values are means.

Estimation of total blood volume

The total blood volume at the baseline was approximately about 7% of body weight. In this study, the total blood volume after hemodilution was estimated using the concept of red blood cells (RBCs) conservation. The amount of remaining RBCs after hemodilution was calculated from the difference between the amount of RBCs at baseline (RBC_{BL}) and the amount of RBCs withdrawn during blood exchange (RBC_E). The estimated blood volume (EBV) was calculated from the amount of remaining RBCs and the final Hct as equation 1.

$$EBV = (RBC_{BL} - RBC_E) / Hct \quad (1)$$

Data analysis of cardiac function

Cardiac function data were analyzed with PVAN software (version3.6, Millar Instruments, TX). Measured cardiac function parameters such as heart rate (HR), end-systolic pressure (P_{es}), end-diastolic pressure (P_{ed}), end-systolic volume (V_{es}) and end-diastolic volume (V_{ed}) were obtained from the pressure and volume signals. Indices of cardiac function were calculated including maximum rate of pressure change (dP/dt_{max}), minimum rate of pressure change (dP/dt_{min}), relaxation time constant (Tau), cardiac out (CO), stroke work (SW) and stroke volume (SV). The values of studied cardiac function indices were averaged from selected 8-12 cardiac cycles at each time point. Furthermore, systemic vascular resistance (SVR) was calculated from MAP and CO to evaluate vascular function.

Statistical analysis

Results are presented as mean ± standard deviation (SD) unless otherwise noted. Data between interested time points in a same group was analyzed using analysis of

variance for repeated measurements (ANOVA) and followed by post hoc analyses with the Dunnett's multiple comparison tests. An unpaired t-test with two-tailed was performed to compare between groups at the time point of interest. All statistics were calculated using GraphPad Prism 4.01 (GraphPad Software, San Diego, CA). Results were considered statistically significant if $p<0.05$.

Results

A total of 22 animals were entered into the study, they were randomly assigned to the following groups: Dx2M (n=6; 67.8±4.2 g); Dx70 (n=8; 67.9±2.0 g); and PEG-HSA (n=8; 66.7±1.7 g). All animals tolerated the hemodilution protocol for the entire period of experiment.

Systemic parameters

Figure 4.2A presents MAP at the baseline and during 60 min after hemodilution with test solutions. Hemodilution drastically decreased MAP by 16%, 21% and 22% in the Dx2M, Dx70 and PEG-HSA groups at 60 min post-hemodilution relative to baseline, respectively ($p<0.05$). In addition, there was a significant difference in MAP between a group hemodiluted with Dx2M and a group hemodiluted with PEG-HSA at the end of experiment (HD60). HR in all groups tentatively decreased over the observation time but only Dx2M and Dx70 caused, at 60min after hemodilution, a significant drop compared to baseline ($p<0.05$, Figure 4.2B). Systemic Hct in all groups remarkably reduced by 46-48% after exchange ($p<0.05$).

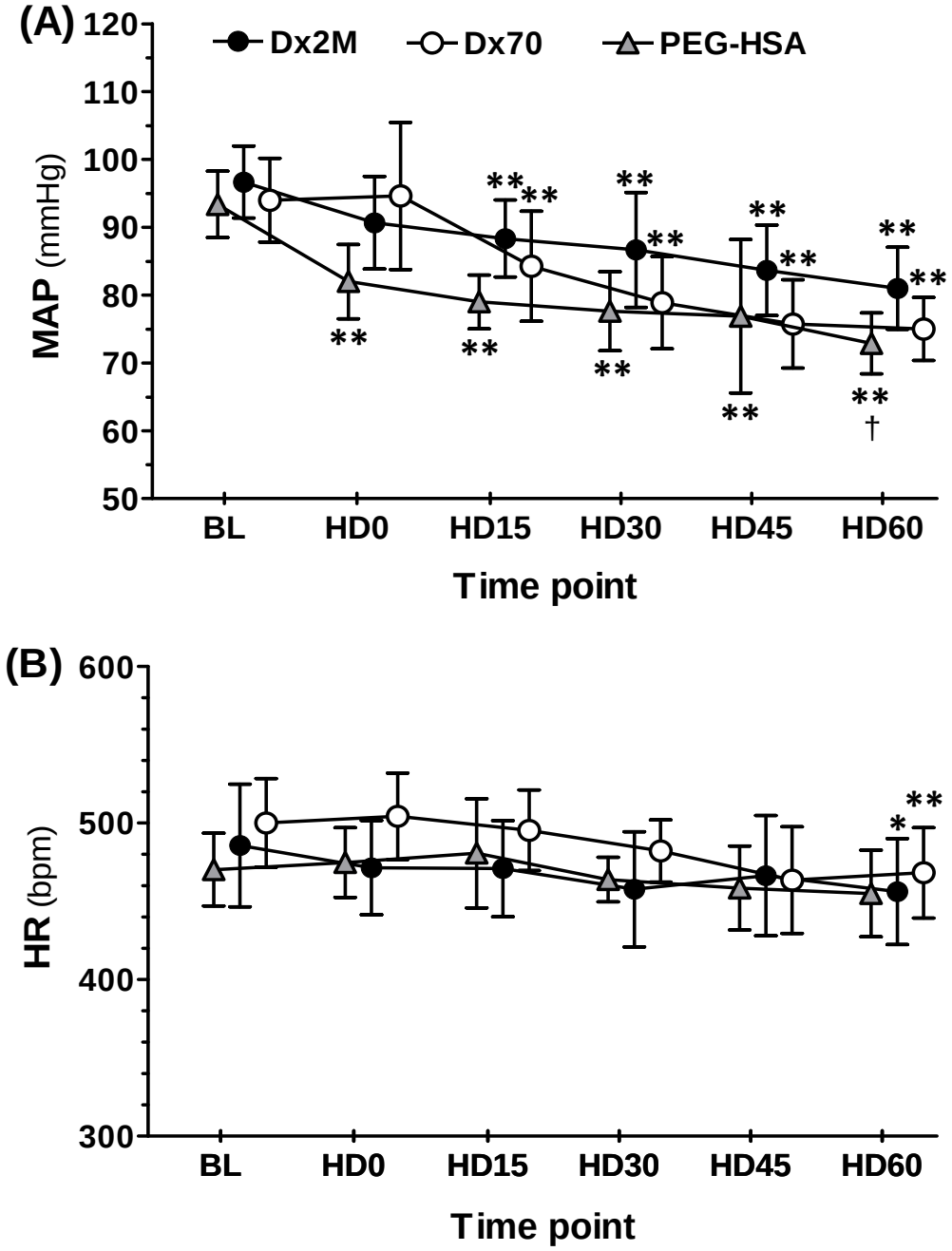

Figure 4.2: (A) Mean arterial pressure (MAP) and (B) Heart rate (HR) measured at baseline (BL), at 0, 15, 30, 45 and 60 minutes after hemodilution (HD0, HD15, HD30, HD45, HD60). Values are presented as means ± SD. * $p<0.05$; ** $p<0.01$ compared with baseline in a same group. † $p<0.05$ compared with the Dx2M group

Viscosity of blood and plasma

Table 4.2 compares viscosity of blood and plasma at 60 min after hemodilution. Dx2M reduced blood viscosity to 77% of the baseline, while Dx70 and PEG-HSA decreased blood viscosity to 66% and 60% of the baseline, respectively. Furthermore, Dx2M significantly increased plasma viscosity for 62% and 75% higher than Dx70 and PEG-HSA, respectively ($p<0.05$).

Cardiac function

Table 4.3 presents left ventricular cardiac indices at the baseline and 60 min after hemodilution. In all groups, hemodilution significantly decreased P_{es} compared to baseline as well as dP/dt_{min} ($p<0.05$). Only animals hemodiluted with Dx2M did not have a significant drop in dP/dt_{max} compared with baseline. Although both Dx2M and PEG-HSA enhanced V_{ed}, only PEG-HSA significantly enhanced V_{ed} at 60min after hemodilution relative to baseline. All test solutions improved SV especially Dx2M and PEG-HSA. The relaxation time constant particularly increased after hemodilution with all test solutions ($p<0.05$). All test solutions also enhanced CO over the baseline value but only Dx2M and PEG-HSA significantly improved, at 60 min after hemodilution, CO compared to baseline ($p<0.05$; Figure 4.3A). Furthermore, PEG-HSA markedly caused CO by 16% higher than Dx70 ($p<0.05$). By overall, SW elevated after hemodilution with the test solutions and was higher than the baseline over the entire observation period. At 60 min after hemodilution, Dx2M significantly increased SW compared with Dx70 ($p<0.05$; Figure 4.3B). The work done by the heart per ejected volume (SW/SV) in the Dx2M group slightly dropped when compared with baseline (by 5%), while this ratio

significantly decreased relative to baseline by 12% in the Dx70 and PEG-HSA groups (Figure 4.4A).

Systemic vascular resistance

Figure 4.4b demonstrates that all test solutions potentially lowered SVR compared to baseline ($p<0.05$). At 60 min after hemodilution, PEG-HSA also significantly decreased SVR compared with Dx70 ($p<0.05$).

Estimated blood volume

All test solutions markedly increased blood volume after hemodilution in range of 27-41% ($p<0.05$; Figure 4.5). However, no significant difference in estimated blood volume between groups was found at the end of experiments.

Table 4.2: Rheological properties at 60 min after hemodilution

60 min after hemodilution	n	Hct (%)	Blood viscosity (cP)	Plasma viscosity (cP)	Plasma COP (mmHg)
Blood (Baseline)	3	52±1	4.7±0.6	1.1±0.1	16±2
Dx2M	6	27±2***	3.6±0.2**	2.1±0.1***	18±2
Dx70	8	28±2***	3.1±0.1***	1.3±0.3***,†††	16±1
PEG-HSA	8	27±2***	2.8±0.2***,†	1.2±0.3**,†††	18±2

Viscosity was measured at shear rate of 160 s^{-1} at 37^{o}C. Values are means. ** p<0.01; *** p<0.001 compared with baseline, † p<0.05; ††† p<0.001 compared with Dx2M.

Table 4.3: Cardiac function indices measured and derived from a pressure-volume (PV) measurement

Parameter	Baseline			60 min after hemodilution		
	Dx2M	**Dx70**	**PEG-HSA**	**Dx2M**	**Dx70**	**PEG-HSA**
P_{es}, mmHg	118.5±6.2	119.0±9.1	113.2±4.1	105.4±9.7 **,‡	103.9±8.6**,‡	91.2±7.9**
P_{ed}, mmHg	7.6±4.1	6.5±1.8	5.5±1.6	7.6±1.9	8.9±3.1	7.8±3.2*
V_{es}, ml	16.5±10.5	13.1±6.9	4.8±2.7	16.1±11.3	8.5±4.9	6.8±3.3
V_{ed}, ml	35.6±10.5	28.0±10.2	22.8±4.7	41.5±13.2	26.0±6.9	28.7±7.8*
SV, ml	20.6±3.8	18.1±5.7	20.2±5.5	27.7±8.3**	21.4±8.0*	25.3±5.4**
dP/dt_{max}, mmHg/s	12,810±1,194	14,305±1,454	13,391±1,882	12,045±1,460	11,768±1,242**	10,977±1,112**
dP/dt_{min}, mmHg/s	-13,981±1,470	-13,021±2,993	-11,614±1,316	-9352±1770**	-9,821±1,628**	-9,236±1,961*
Tau-Weiss, ms	6.2±1.0	5.8±0.8	6.1±0.5	7.3±1.3**	6.7±1.1*	7.0±0.6*

Values are presented as means ± SD. HR, heart rate; P_{es}, end systolic pressure; P_{ed}, end diastolic pressure; V_{es} end systolic volume; V_{ed}, end diastolic volume; SV, stroke volume; dP/dt_{max}, maximum rate of pressure change; dP/dt_{min}, minimum rate of pressure change; Tau, relaxation time constant. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared with baseline. ‡ $p<0.05$ compared with the PEG-HSA group.

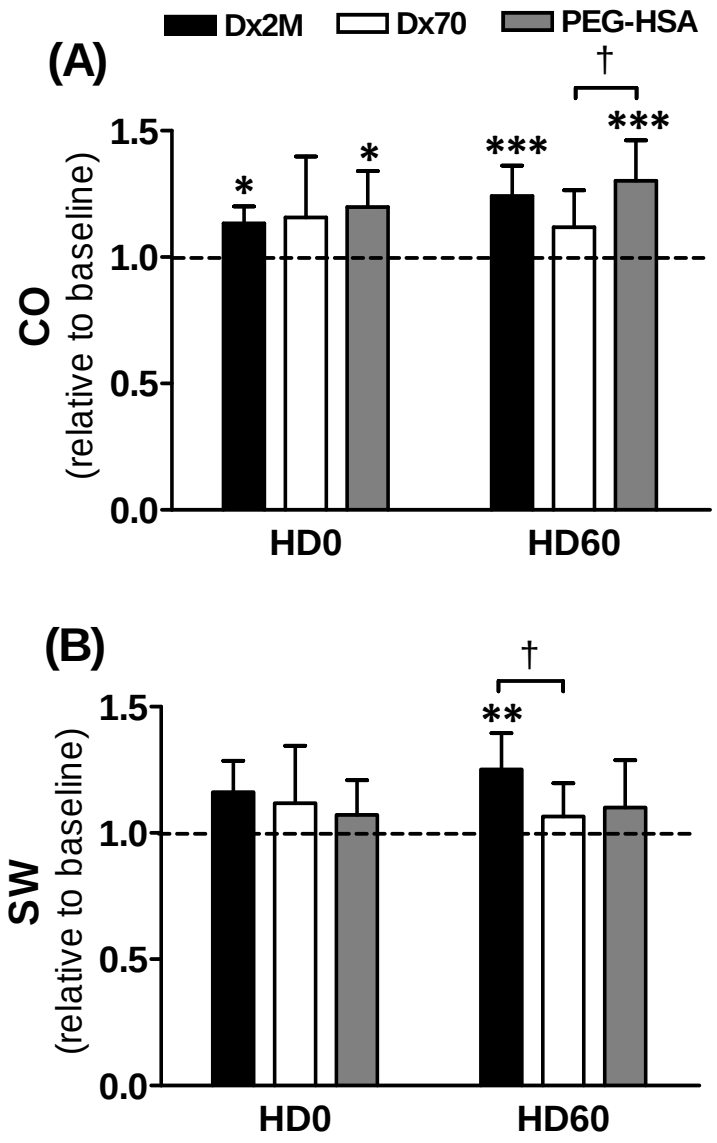

Figure 4.3: (A) Cardiac output (CO) and (B) Stroke work (SW) at 0 min and 60 min after hemodilution. Broken line represents the values at baseline. Values are presented as means ± SD. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared with baseline. † $p<0.05$ compared between groups.

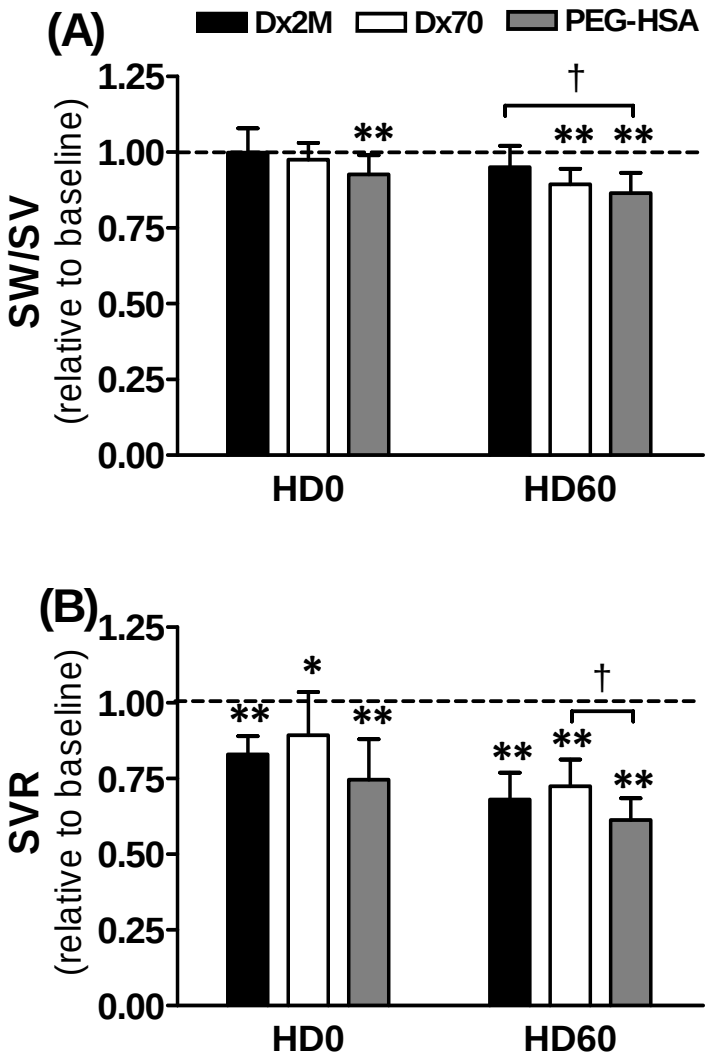

Figure 4.4: (A) Work done by the heart per stroke volume (SW/SV ratio) and (B) Systemic vascular resistance (SVR) at 0 min and 60 min after hemodilution. Broken line represents the values at baseline. Values are presented as means ± SD. * $p<0.05$; ** $p<0.01$ compared with baseline. † $p<0.05$ compared between groups

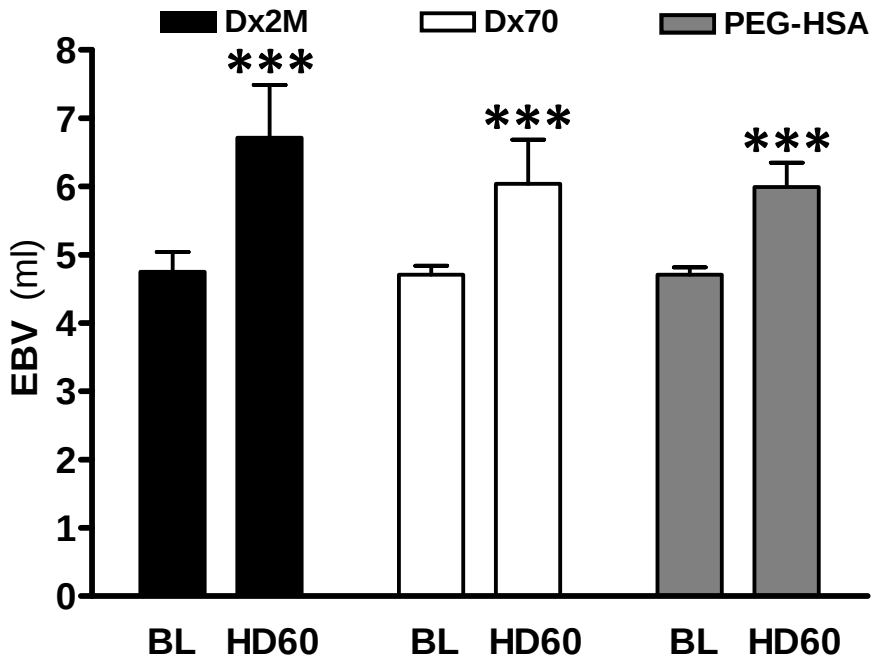

Figure 4.5: Estimated blood volume (EBV) calculated from remained RBC volume at 60 min after hemodilution compared with baseline. Values are presented as means ± SD. *** $p<0.001$ compared with baseline.

Discussions

The main finding of this study is that, in an acute isovolemic hemodilution (Hct 28%) and under anesthesia, PEG-HSA (low viscogenic PE) particularly affected on cardiac function as similar as Dx2M (high viscogenic PE) and better than Dx70 (low viscogenic PE). Furthermore, PEG-HSA significantly lowered SVR compared with other test PEs. PEG-HSA potentially had beneficial characteristics of PE on the heart performance due to significantly improved CO, SV and lowered SW/SV ratio, although MAP decreased. Our results also suggest that the beneficial effects of PEG-HSA on cardiac and vascular function were not mainly caused by its viscosity which is obviously unlike Dx2M.

The rheological properties of blood depend on RBC concentration, RBC aggregation, plasma viscosity and cell deformity. Our results show that an increase of plasma viscosity by viscogenic PEs enhanced blood viscosity toward the normal value, although the Hct decreased. Blood viscosity is a significant factor for endothelial wall shear stress as well as wall shear rate. Cabrales et al. performed microcirculation study in an extreme hemodilution model and found that PEG-HSA caused higher arteriolar and venular wall shear stress than Dx70, although PEG-HSA had lower viscosity than Dx70 [6, 7]. Their results numerically showed that PEG-HSA caused higher wall shear stress because it provided higher wall shear rate and microvascular blood flow compared to Dx70. Their results supported our finding because, in our study, PEG-HSA significantly enhanced CO relative to Dx70, implying that PEG-HSA improved blood perfusion better than Dx70. Shear stress is a major determinant for the production of vasodilation mediators such as nitric oxide and prostaglandin [10, 11, 22]. Therefore, PEG-HSA and

Dx2M dilated blood vessel more effective than Dx70 due to their higher wall shear stress. This conclusion is also supported by Cabrales et al.'s study which reported that PEG-HSA increased arteriolar and venular diameters greater than Dx70 [7]. Vasodilation in the circulatory system leads to a decrease in SVR and blood pressure. PEG-HSA and Dx2M decreased SVR lower than Dx70 during the observation period, suggesting that PEG-HSA and Dx2M possibly induced more production of vasodilators than Dx70.

The important characteristic of plasma expanders is a volume expansion. The high colloid osmotic pressure of PEs results in shifting of fluids from extravascular space into intravascular space. We calculated the EBV after hemodilution by using the concept that RBCs should be conserved after hemodilution. Our calculations show that blood volume significantly increased relative to baseline after hemodilution for all PEs. EBV after hemodilution may be under estimation because we did not take account of the ratio of whole-body to large-vessel hematocrit (F-cell ratio) after hemodilution.[9] However, taking account of F-cell ratio does not change the tendency of EBV.

The load-dependent parameters derived from pressure-volume measurement (dP/dt_{max} and dP/dt_{min}) and P_{es} decreased after hemodilution, suggesting the sign of the depression of cardiac function in systole and diastole. However, this depression is controversial with an increase of CO. At 60min after hemodilution, Dx70 and PEG-HSA significantly reduced dP/dt_{max} relative to baseline while Dx2M did not show that. These results indicate that systolic function was more deficient when hemodilution with Dx70 and PEG-HSA. The decrease of P_{es} in all groups was in accompanied with the reduction of mean arterial blood pressure. Furthermore, the decrease of P_{es} may be the result of lowering of SVR and afterload. Our study also evaluated the fall of left ventricular

pressure during isovolumic relaxation phase using the Weiss relaxation time constant (Tau). We found that hemodilution with each test PEs increased Tau, indicating a prolong diastolic relaxation and a sign of diastolic function impairment [13]. In the isovolumic relaxation phase, there is ATP usage for calcium uptake by sarcoplasmic reticulum. Thus, hemodilution might delay this process due to an increased Tau.

As the oxygen carrying capacity decreases in hemodilution, the compensatory mechanisms increase the volume flow rate to maintain or sustain the oxygen delivery for tissue oxygenation with limitation. Our findings present that PEG-HSA potentially enhanced and maintained CO higher than the baseline over the observation period as similar as Dx2M, although PEG-HSA had significantly lower viscosity than Dx2M. In our study, HR after hemodilution was relatively lower than the baseline; therefore, the enhancement of CO after hemodilution with PEG-HSA, Dx2M or Dx70 was the result of an increase of SV. Furthermore, PEG-HSA remarkably increased an end-diastolic volume (V_{ed}) while an end-systolic volume (V_{es}) was not significantly changed at 60 min after hemodilution, resulting in the increase of SV. The increase of V_{ed} or preload after hemodilution also caused the heart to work more as shown by the increase of stroke work. Our results further present that an increase of plasma viscosity with Dx2M led to an increase of stroke volume and more work or energy required to pump out blood, unlike PEG-HSA performed. Microvascular studies showed that increase of CO correlated to increase of microvascular blood perfusion and oxygen delivery and extraction [5, 15, 25]. In addition, Cabrales and coworkers observed that PEG-HSA increased microvascular oxygen delivery and extraction higher than Dx70 in an extreme hemodilution model [7]. Regarding to their findings, we concluded that, in our study, hemodilution with PEG-

HSA and Dx2M provided sufficient oxygen for the heart to perform work and this was better than hemodilution with Dx70.

In our experiments, the blood conductance at the baseline was estimated from the baseline Hct and was used to calculate the volume-related indices such as V_{es}, V_{ed}, SV and SW. This method might give an over or under estimation on V_{es} and V_{ed}. However, this method did not significantly affect on the differential volume-related indices such as SV and SW. Furthermore, the parallel conductance (V_p) determined by a bolus of hypertonic saline injection was not a constant for each animal after hemodilution with PEs. This is another cause that may affect on the accuracy of the volume-related cardiac function indices. To minimize the error from the V_p measurement, a bolus of hypertonic saline was injected when the heart rhythm was regular and the linear regression correlation coefficient of the extrapolation to determine V_p was higher or equal to 0.8 ($r \geq 0.8$).

We conclude that PEG-HSA has a significant lower in viscosity compared to other higher viscogenic PEs but it efficiently improves cardiac output and preload and lowers pumping work per stroke volume after hemodilution. These beneficial effects of PEG-HSA are not the direct results of increase of plasma viscosity, unlike Dx2M and Dx70. Therefore, PEG-HSA possibly has the interaction mechanisms with vasculature different from higher viscogenic PEs but it causes the similar effects on cardiovascular system, leading to an interesting issue on its interaction mechanism for a further study of this novel PE.

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Chapter 5

The Increased Vascular Nitric Oxide Enhances Ventricular Function in Acute Isovolemic Hemodilution with Low Viscogenic Plasma Expander

Abstract

Nitric oxide (NO) is a critical endothelial-derived relaxing factor synthesized from endothelial NO-synthase, playing a role in the control of vascular homeostasis, inhibition of vascular smooth muscle cells proliferation and modulation of vascular tone and vascular dilation. NO also affects the function of various organs, including the myocardium. Elevated plasma viscosity induces sustained NO-mediated dilation due to increased vascular wall shear stress and increases perivascular NO concentration and microvascular perfusion. We hypothesized that increased shear stress-induced NO has beneficial effects on cardiac function during hemodilution as a result of elevated plasma viscosity. In this study, acute isovolemic hemodilution was performed as a study protocol in pentobarbital-anesthetized hamsters. DETA NONOate was used as NO donor during hemodilution. To test our hypothesis, one group of animals was hemodiluted with dextran 70 kDa (Dx70; low viscosity plasma expander, 3.0 cP) followed by 0.1 ml of 0.01 M NaOH (vehicle) as a control group, and another group was administered with 0.1 ml of 10 mM DETA NONOate during hemodilution with Dx70. Left ventricular cardiac function was determined from pressure-volume (PV) measurements obtained with a miniaturized PV conductance catheter. Experimental results showed that the effects of exogenous NO on systemic and cardiac function parameters were not long-lasting in the group infused with NO donor, being over 10-15 min after NO donor injection. Cardiac

output increased 20-22% and 6-9% in NO donor and vehicle groups, respectively. Stroke work was unchanged compared to baseline in both groups. During 20 min after hemodilution, stroke work per stroke volume was significantly lower in the NO donor group compared with the vehicle group ($p<0.05$). The maximum rate of pressure change (dP/dt_{max}) was not different between groups but the minimum rate of pressure change (dP/dt_{min}) was significantly lower in the NO donor group compared with the control group ($p<0.05$). Systemic vascular resistance (SVR) in the NO donor group reduced to 55-62% of the baseline ($p<0.05$) whereas SVR in the vehicle group decreased to 80-92% of the baseline ($p<0.05$). In addition, lower reduction of SVR by NO donor was evidenced by greater vasodilation of both arterioles and venules observed in the skin fold window chamber. These preliminary findings demonstrated the beneficial effects of supplemented NO on cardiac function in an acute hemodilution with low viscosity plasma expander, in part providing the evidence to support that endogenous NO induced by wall shear stress, due to increased plasma viscosity, has positive effects on cardiac function.

Keywords: nitric oxide; hemodilution; cardiac function; vascular resistance; conductance catheter; plasma viscosity

Introduction

Vascular wall shear stress on the endothelial cells is a dependent factor modulated by changing of blood or plasma viscosity and wall shear rate. Shear stress plays an important role in endothelial function including endothelium-derived relaxing factors (EDRF) release, endothelial gene expression, endothelial cells reorganization and secretion of anticoagulants [2, 10, 14, 23, 24, 34]. Nitric oxide (NO) is a critical EDRF synthesized from endothelial NO-synthase (eNOS), playing a role in the control of vascular homeostasis, inhibition of vascular smooth muscle cells proliferation, modulation of vascular tone and vascular dilation [6, 11, 21, 27].

Microvascular studies in a hamster window chamber have exhibited that increased plasma viscosity with high viscogenic plasma expanders (HVPEs) effectively caused higher wall shear stress and vasodilation compared to low viscogenic plasma expanders (LVPEs) during acute hemodilution [4, 5, 18]. Elevated plasma viscosity also induces sustained NO-mediated dilation and increases perivascular NO concentration and microvascular perfusion [8, 33]. Therefore, an improvement of microvascular function when hemodilution with HVPEs correlates to an increase of NO, suggesting a parallel improvement in cardiac function.

A role of NO on the regulation of cardiac function and circulatory system has been studied in both normal and pathological heart. Some studies reported that NO had a bimodal effects on cardiac inotropy; positive inotropic effect at low amounts of NO exposure and negative inotropic effect at higher amounts [3, 17, 29]. However, several studies found no inotropic effect of both endogenous and exogenous NO at the baseline condition [15, 30]. In spite of many studies about the effects of NO on cardiac function,

it is still complex and unclear. In the past, cardiac function studies were performed in an isolated heart or cardiomyocytes but theses experimental setups did not truly represent physiological conditions, unlike an in vivo study. However, a real-time ventricular volume measurement has been problematic issue in an *in vivo* study. Recently, a miniaturized pressure-volume (PV) conductance catheter promisingly provides the simultaneous measurement of ventricular pressure and volume, leading to a powerful approach to quantify cardiac function in small animals [25, 26, 32].

This study tested hypothesis that the supplement of exogenous NO during an acute hemodilution with LVPE provides beneficial effects on cardiac function as the finding with an increase of shear stress dependent-released NO during hemodilution with HVPE. Left ventricular cardiac function indices derived from the pressure-volume measurement using a miniaturized PV conductance catheter were quantitatively analyzed under conditions of acute isovolemic hemodilution (28% hematocrit). In addition, the effect of exogenous NO on vascular relaxation was also determined by vascular diameter measurement using a skin fold window chamber.

Methods and Materials

Animal preparation for cardiac function measurement

Studies were performed in anesthetized male Golden Syrian hamsters (Charles River Laboratories; Boston, MA) weighing 60-70g. Animal handling and care followed the NIH Guide for Care and Use of Laboratory Animals. The experimental protocol was approved by the local animal care committee. Surgery was performed following i.p. injection of sodium pentobarbital (50mg/kg). The left jugular vein was catheterized to

allow fluid infusion and left femoral artery was cannulated for blood pressure monitoring and blood withdrawal and sampling. Furthermore, animal was performed tracheotomy and cannulated with a polyethylene-90 tube to facilitate spontaneous breathing. Animal was placed in the supine position on the heating pad to maintain the body temperature at 36-37^{o}C. During experiment, if animals response to a toe pinching, a small bolus of sodium pentobarbital (10-15mg/kg, i.p.) will be given.

Animal preparation for a skin fold window chamber

The hamster skin fold window chamber model is widely used for microvascular studies in the unanesthetized state, and the complete surgical technique for the preparation has previously been described in detail [33]. The animal was allowed at least 2 days for recovery; its chamber was then assessed under the microscope for any signs of edema, bleeding, or unusual neovascularization. Arterial and venous catheters (polyethylene-50) were implanted in the carotid artery and jugular vein, respectively. Catheters were tunneled under the skin and exteriorized at the dorsal side of the neck.

Inclusion criteria

Animals under anesthesia were suitable for the experiments if 1) animals had no bleeding and 2) systemic parameters were within normal range, namely, mean arterial pressure (MAP) above 80 mmHg, heart rate (HR) above 320 beats/minute and systemic hematocrit (Hct) above 45%.

Systemic parameters

The MAP was monitored continuously (MP150, Biopac System Inc., Santa Barbara, CA), except during blood exchange. The Hct was determined from centrifuged

arterial blood samples taken in heparinized capillary tubes (Readacrit Centrifuge, Clay Adams, Parsippany, NJ).

Isovolemic hemodilution protocol

Anesthetized hamsters were exchanged by 40% of estimated total blood volume (BV) with Dextran 70 kDa (Dx70; viscosity 3.0 cP), lowering systemic Hct by 45%. Total BV was estimated as 7% of body weight. Dx70 was infused into the left jugular vein catheter at a rate of 0.1 ml/min with simultaneous blood withdrawal at the same rate from the left femoral artery catheter by using a dual syringe pump (33 syringe pump, Harvard Apparatus, Holliston, MA). Blood sample was collected at the end of experiment for blood conductance measurement. Animals were monitored for 50min after complete hemodilution. Systemic parameters (MAP and Hct) were recorded and analyzed at baseline and after hemodilution. Figure 5.1 illustrates the experimental protocol.

Cardiac function measurement

Closed chest method was performed to assess cardiac function in this study [26]. The right common carotid artery was exposed allowing a 1.4F pressure-volume conductance catheter (PV catheter; SPR-839, Millar Instruments, TX) to be inserted. The PV catheter was advanced passing through the aortic valve into the left ventricle. At the baseline and the end of experiment, a bolus of 15% hypertonic saline (10 μl) was intravenously injected to determine the parallel volume (V_p) [1]. The pressure and volume signals were instantaneously digitized and acquired (MPVS300, Millar Instruments, Houston, TX and PowerLab 8/30, ADInstruments, Colorado Springs, CO). Cardiac function data were analyzed with PVAN software (version3.6, Millar

Instruments, TX). The values of studied cardiac function indices were averaged from selected 8-12 cardiac cycles at each time point.

Vascular diameter measurement

Anesthetized animals were placed in a restraining tube with a longitudinal slit from which window chamber protruded and then were fixed to the microscopic stage of a transillumination intravital microscope (BX51WI; Olympus, New Hyde Park, NY). The tissue image was projected onto a charge-coupled device camera (COHU 4815), connected to a videocassette recorder (AG-7355, JVC) and viewed on a monitor. Measurements were carried out using a ×20 water immersion objective (UMPlanFI 20×/0.50 , Olympus). A video image-shearing method was used to measure the vessel diameter at the baseline, 10min after hemodilution and 5min after NO donor administration [12].

Vascular resistance

Systemic vascular resistance (SVR), the resistance to flow in the periphery circulation, was mathematically calculated from the measured values as SVR =MAP/CO, where MAP is the mean arterial pressure and CO is cardiac output. Microvascular resistance (MVR) defines the resistance to flow in the small arterioles and venules. MVR is a dependent variable on blood viscosity (μ) and vascular geometry: length (L) and diameter (D). In this study, the length of vessel was assumedly constant. Therefore, MVR was proportional to viscosity and inversely proportional to the 4^{th} power of diameter ($MVR \propto \mu/D^4$).

Nitric oxide (NO) donor dose response

Diethylenetriaamine NONOate (DETA NONOate; $C_4H_{13}N_5O_2$, Cayman Chemical, Ann Arbor, MI) was used as NO donor. DETA NONOate is highly soluble in water, liberates 2 moles of NO per mole of parent compound and has a 20 hrs half-life in physiological buffer at 37^{o}C [13]. DETA NONOate was prepared in a stock solution (0.01 M NaOH) for three different concentrations (25, 10 and 1 mM). At 20 min after hemodilution, NO donor dosage was administered by a bolus injection (0.1ml i.v.).

Experimental groups

Animals were randomly divided into two groups. A first group was hemodiluted with Dx70 and administered with 0.1 ml of 0.01M NaOH (group labeled Vehicle). A second group was hemodiluted with Dx 70 and administered with 0.1 ml of DETA NONOate solution (group labeled NO donor).

Estimation of left ventricular blood volume

Left ventricular blood volume was measured continuously in conductance units (RVU; relative volume unit) and converted to actual blood volume (μl) at the end of the experiment. The blood conductance at the baseline was estimated from baseline hematocrit, using the pooling data of blood conductance and hematocrit relationship (see Appendix II).

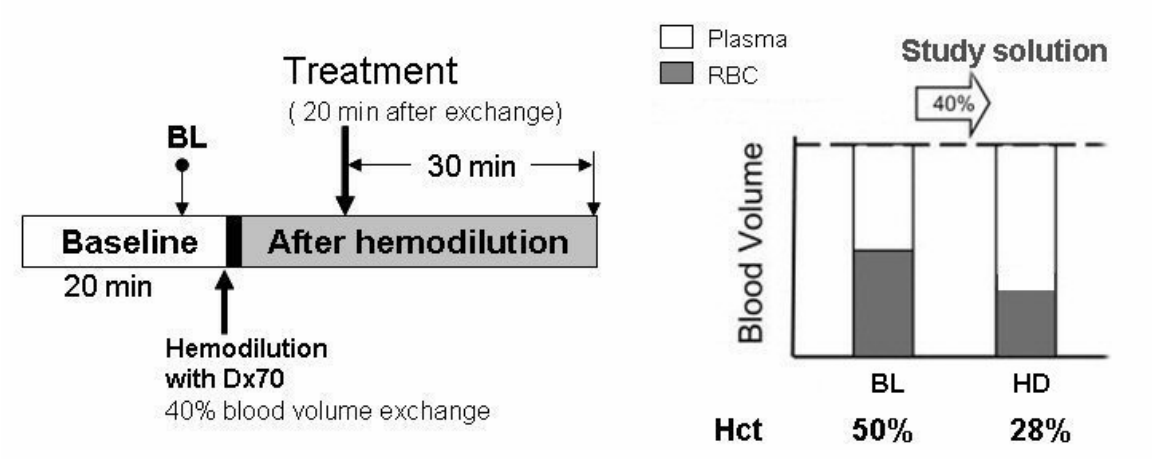

Figure 5.1: Schematic diagram of acute isovolemic hemodilution protocol. A bolus of NO donor was administered (0.1ml i.v.) at 20min after hemodilution. BL, baseline; HD, hemodilution.

Statistical analysis

Results are presented as mean ± standard deviation (SD). All data are presented as absolute values and ratios relative to baseline values unless otherwise noted. Data between interested time points in a same group were analyzed using analysis of variance for repeated measurements (ANOVA) and followed by post hoc analyses with the Dunnett's multiple comparison tests. A two-way ANOVA followed by Bonferroni posttests was performed to compare data between groups. An unpaired t-test was performed to compare the vascular diameters and vascular resistance between groups. All statistics were calculated using GraphPad Prism 4.01 (GraphPad Software, San Diego, CA). Results were considered statistically significant if $p<0.05$.

Results

Animal administered with 25 mM of DETA NONOate had a dramatic drop in MAP (from 85 mmHg at the baseline to 40 mmHg within 2 min after NO donor infusion) and it died within 35min after infusion as shown in Figure 5.2A. For the dose of 10 mM of DETA NONOate, MAP decreased to 72% of the baseline and returned to baseline level within 10-15min after infusion (Figure 5.2B). In contrast, the dose of 1 mM of DETA NONOate did not caused a decrease in MAP after NO donor infusion (Figure 5.2C). It evidenced that using higher or lower dosage was not justified. Therefore, all experiments in a NO donor group were performed with 10 mM DETA NONOate.

Animals were randomly assigned to the experimental groups: vehicle (n=10; 68±2 g) and NO donor (n=10; 67±3 g). Seven animals in each group were used for cardiac function study and three animals in each group were used for vascular diameter

measurement in window chamber. Arterial Hct dropped from 51±2% at baseline to 29±2% and from 52±3% at baseline to 27±3% in the vehicle and NO donor groups, respectively.

Figure 5.3 presents MAP and HR at the baseline, 15 min after hemodilution and during 30 min after treatment. MAP dramatically dropped during 10 min after NO donor infusion and was significantly decreased 24-31% compared to baseline ($p<0.05$), while MAP in the vehicle group dropped 5-15% relative to baseline. HR in the NO donor group increased significantly during 2 min after NO donor administration ($p<0.05$) and continually decreased for the rest of experimental period, while HR in the vehicle group gradually dropped after hemodilution and was significantly lower compared with baseline only at the end of experiment ($p<0.05$).

Cardiac output (CO) increased after hemodilution in both groups as shown in Figure 5.4A. In addition, CO was promisingly enhanced 20-27% during 15 min after NO donor infusion compared with baseline ($p<0.05$). Despite higher CO in the group infused with NO donor, there was no statistically significant difference between groups. Stroke volume (SV) also increased after hemodilution as similar as CO (Figure 5.4B). SV increased 15-23% from the baseline after NO donor infusion, whereas SV increased 7-13% in the vehicle group. Stroke work (SW) was unchanged compared to the baseline in both groups. However, the ratio of SW to SV (SW/SV) was significantly different between groups during 5 min after NO infusion as shown in Figure 5.4C ($p<0.05$). The relationship between SW and CO after treatment is plotted in Figure 5.5. CO linearly correlated with SW and the correlation coefficients were r=0.9562 and r=0.9495 in the vehicle and NO donor groups, respectively.

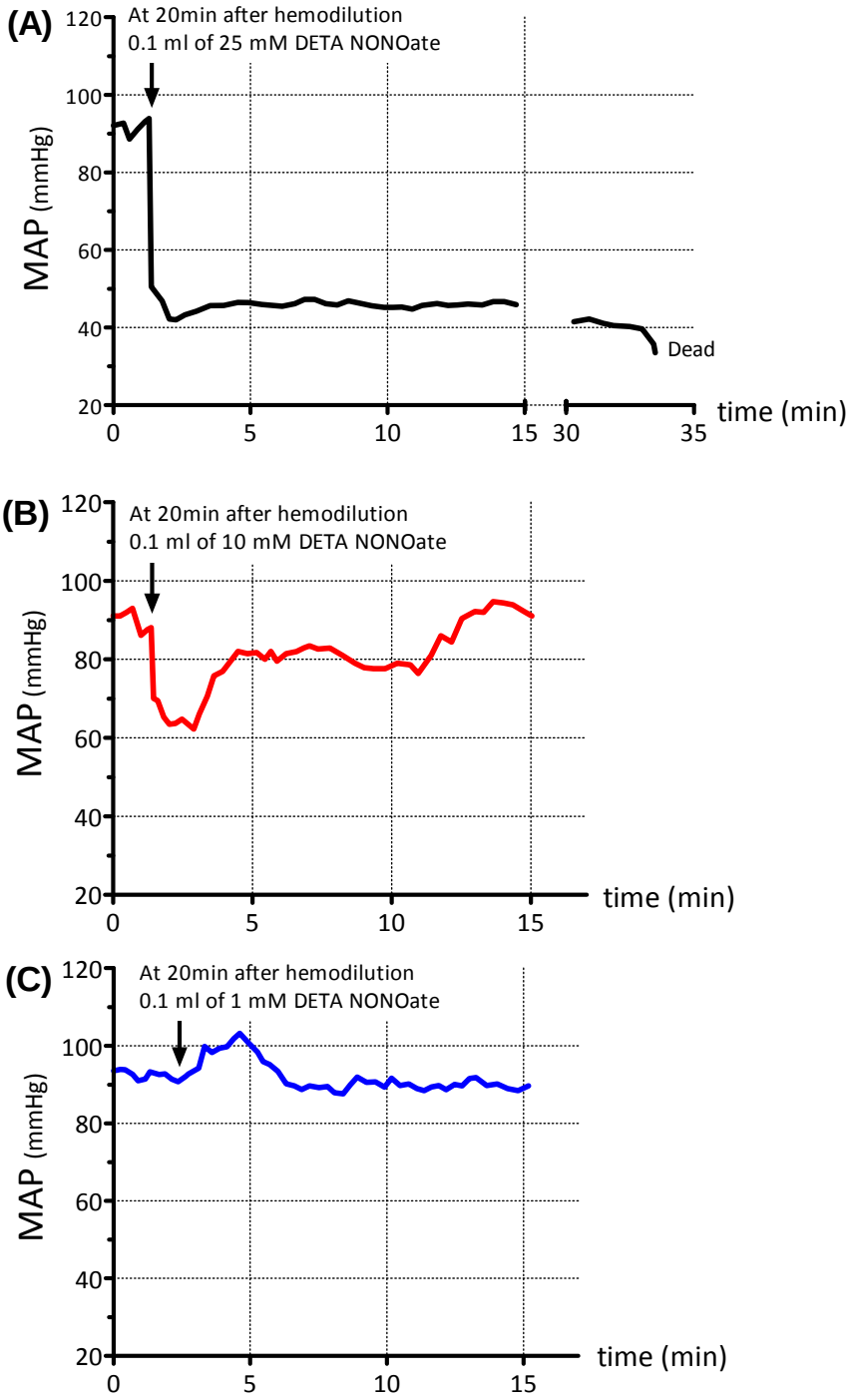

Figure 5.2: Mean arterial pressure (MAP) recorded after DETA NONOate, NO donor, infusion with three different concentrations (25, 10 and 1 mM) to determine the dose-respond. A bolus of NO donor (0.1ml i.v.) was infused at 20 min after hemodilution.

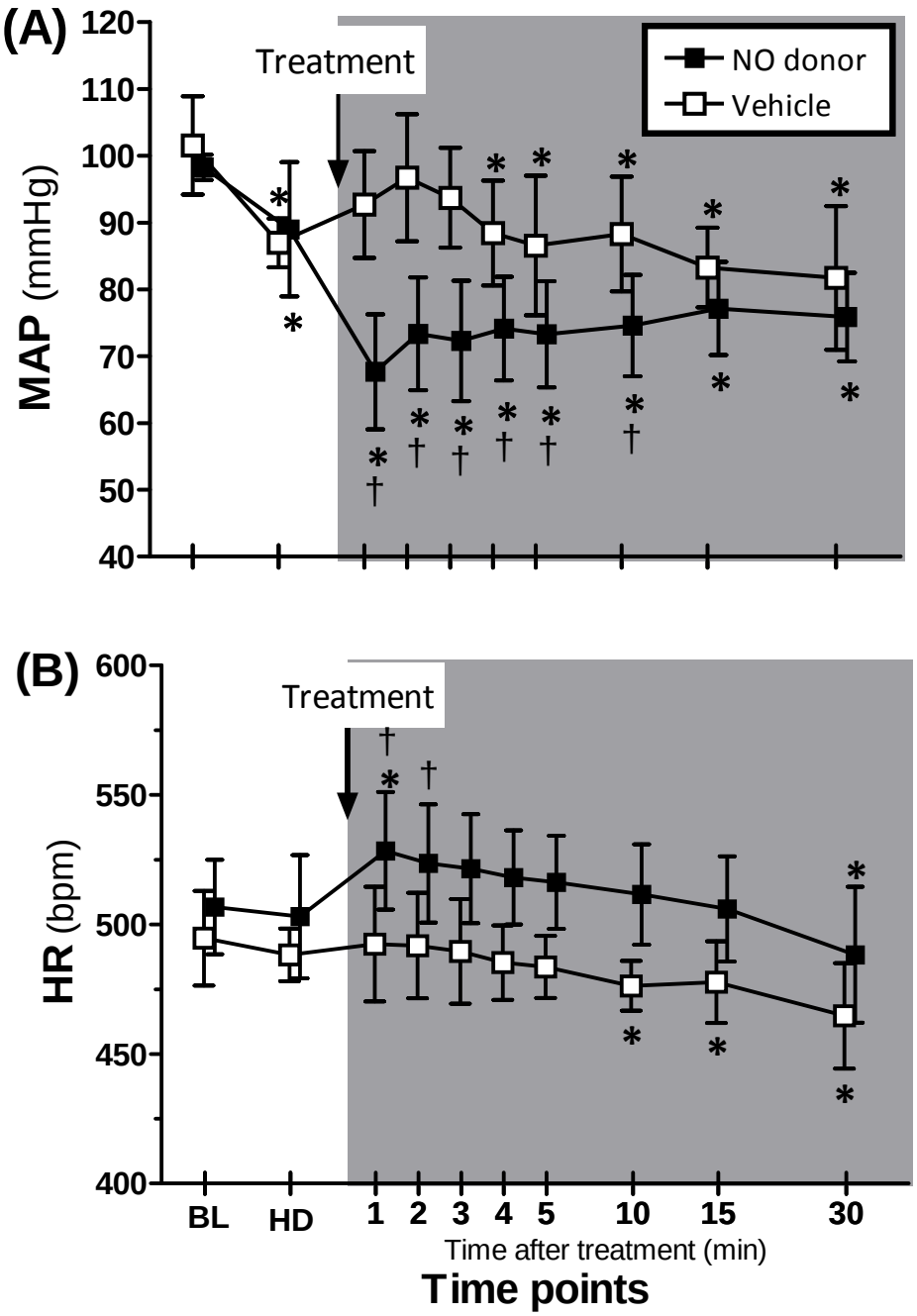

Figure 5.3: (A) Mean arterial pressure (MAP) measured at baseline (BL), at 15 minutes after hemodilution (HD), at 1, 2, 3, 4, 5, 10, 15 and 30 minutes after treatment**.** (B) Heart rate (HR) measured at baseline (BL), at 15 minutes after hemodilution (HD) and at 1, 2, 3, 4, 5, 10, 15 and 30 minutes after treatment. Values are presented as means ± SD. * $p<0.05$ compared with BL. † $p<0.05$ between groups.

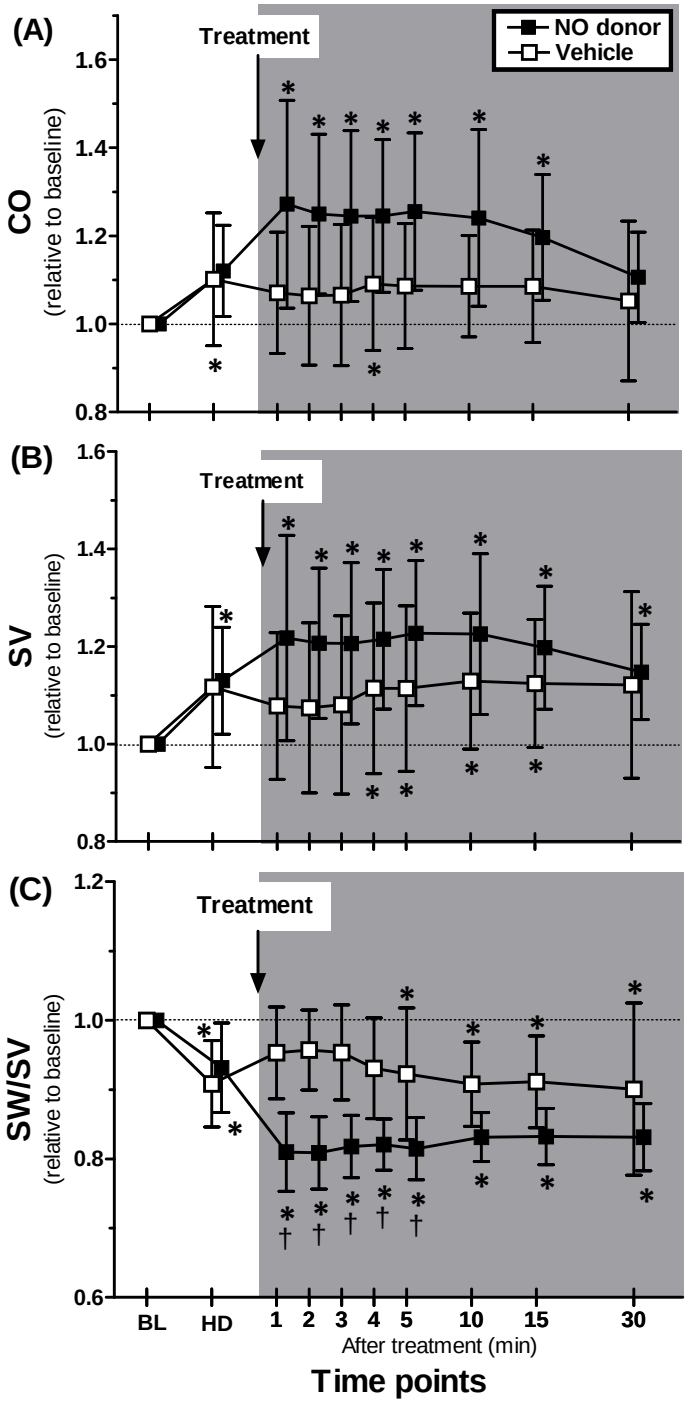

Figure 5.4: Left ventricular function indices derived by PV conductance catheter. **(**A) Cardiac output (CO), (B) Stroke volume (SV) and (C) Stoke work to stroke volume ratio (SW/SV) at baseline (BL), at 15 minutes after hemodilution (HD) and at 1, 2, 3, 4, 5, 10, 15 and 30 minutes after treatment. Values are presented as means ± SD. * $p<0.05$ compared with BL. † $p<0.05$ between groups.

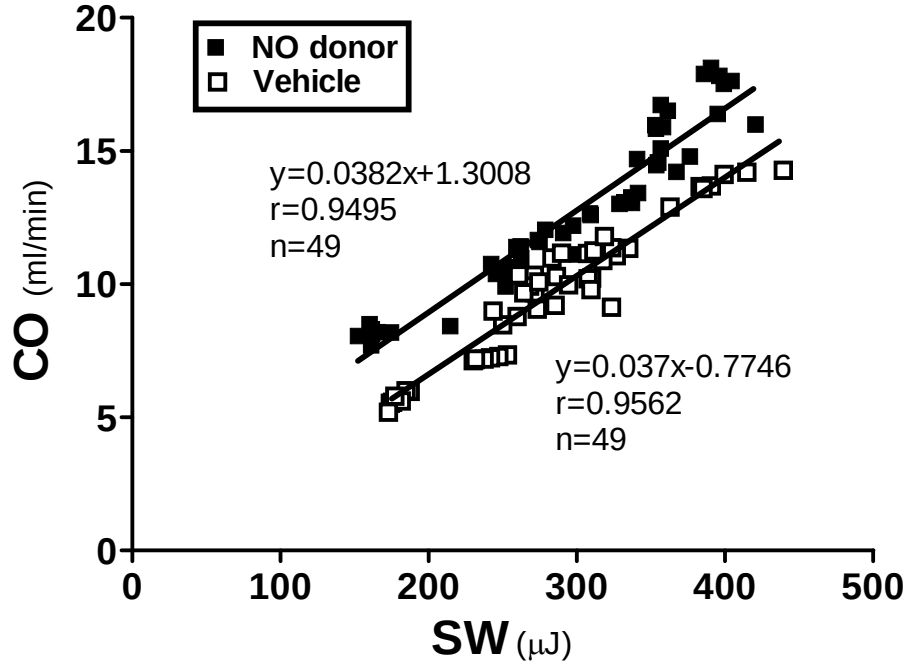

Figure 5.5: Relation between Cardiac output (CO) and stroke work (SW) during 30 min after treatment. Solid lines are a linear regression for each experimental group (Vehicle: y=0.037x-0.7746, r=0.956; NO donor: y=0.0382x+1.3008, r=0.9495).

Figure 5.6 shows cardiac function indices related to left ventricular pressure. End-systolic pressure (P_{es}) significantly decreased in the NO donor group compared with the vehicle group ($p<0.05$) whereas no significant difference between groups was observed in end-diastolic pressure (P_{ed}). The maximum rate of pressure change (dP/dt_{max}) in the vehicle group was not different compared with the NO donor group. In contrast, the minimum rate of pressure change (dP/dt_{min}) drastically decreased (39-44% from baseline) after NO donor infusion and was significantly lower compared with the vehicle group ($p<0.05$). The significant decrease in dP/dt_{min} was also evident in the relaxation phase of pressure-volume loops between at the baseline and 1 min after NO donor infusion as depicted in Figure 5.7. The relationship between end-systolic pressure (P_{es}) and end-systolic volume (V_{es}) is plotted as shown in Figure 5.8. The slope of the relationship between P_{es} and V_{es} represents an end-systolic elastance (E_{es}). It is evident that the NO donor group had relatively higher E_{es} compared with the vehicle group.

Systemic vascular resistance (SVR) is presented in Figure 5.9A. SVR in the NO donor group reduced to 55-62% of the baseline whereas SVR in the vehicle group decreased to 80-92% of the baseline ($p<0.05$). In addition, the lower reduction of SVR by NO donor was further evidenced by higher vasodilation of both arterioles and venules observed in the skin fold window chamber ($p<0.05$; Figure 5.9B). In our previous study, blood viscosity at the baseline (52% Hct) was 4.71±0.62 cP and blood viscosity after hemodilution with Dx70 (28% Hct) was 3.09±0.09 cP. After treatment, MVR in arterioles was significantly lower in the NO donor group compared with the vehicle group ($p<0.05$) whereas MVR in venules was not different between groups (Figure 10).

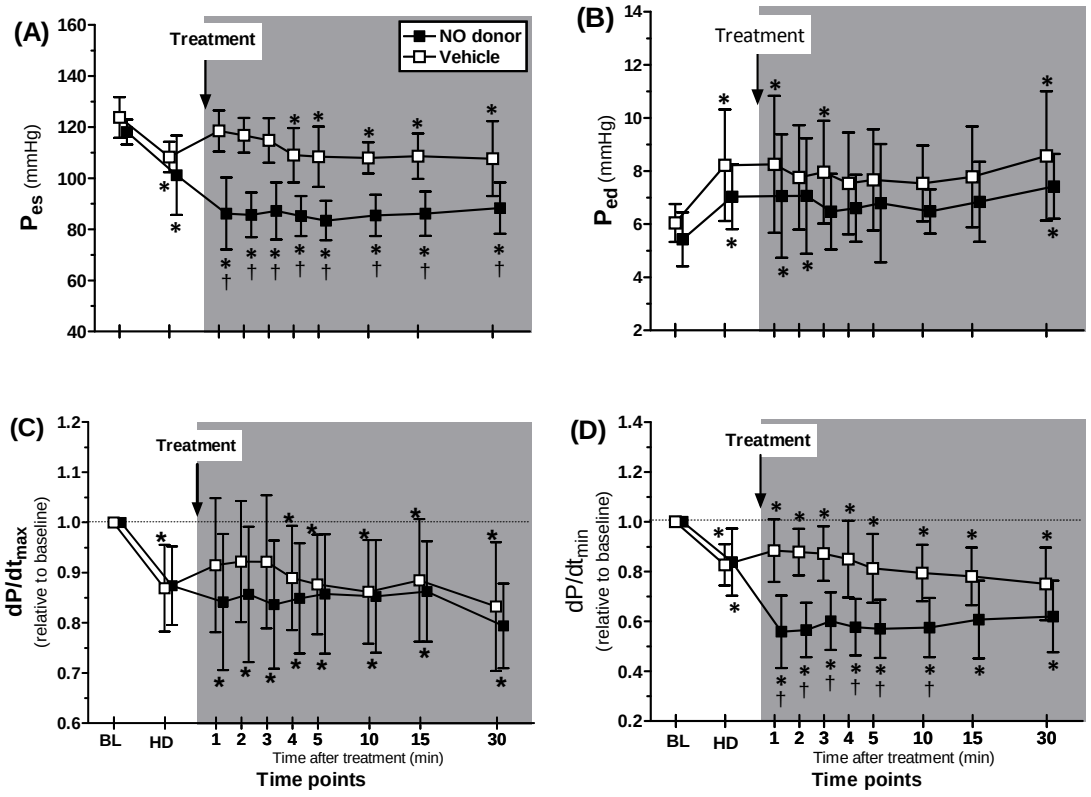

Figure 5.6: Left ventricular function indices related to left ventricular pressure. (A) End-systolic pressure (P_{es}), (B) End-diastolic pressure (P_{ed}), (C) Maximum rate of pressure change (dP/dt_{max}) and (D) Minimum rate of pressure change (dP/dt_{min}) at baseline (BL), at 15 minutes after hemodilution (HD) and at 1, 2, 3, 4, 5, 10, 15 and 30 minutes after treatment. Values are presented as means ± SD. * $p<0.05$ compared with BL. † $p<0.05$ between groups.

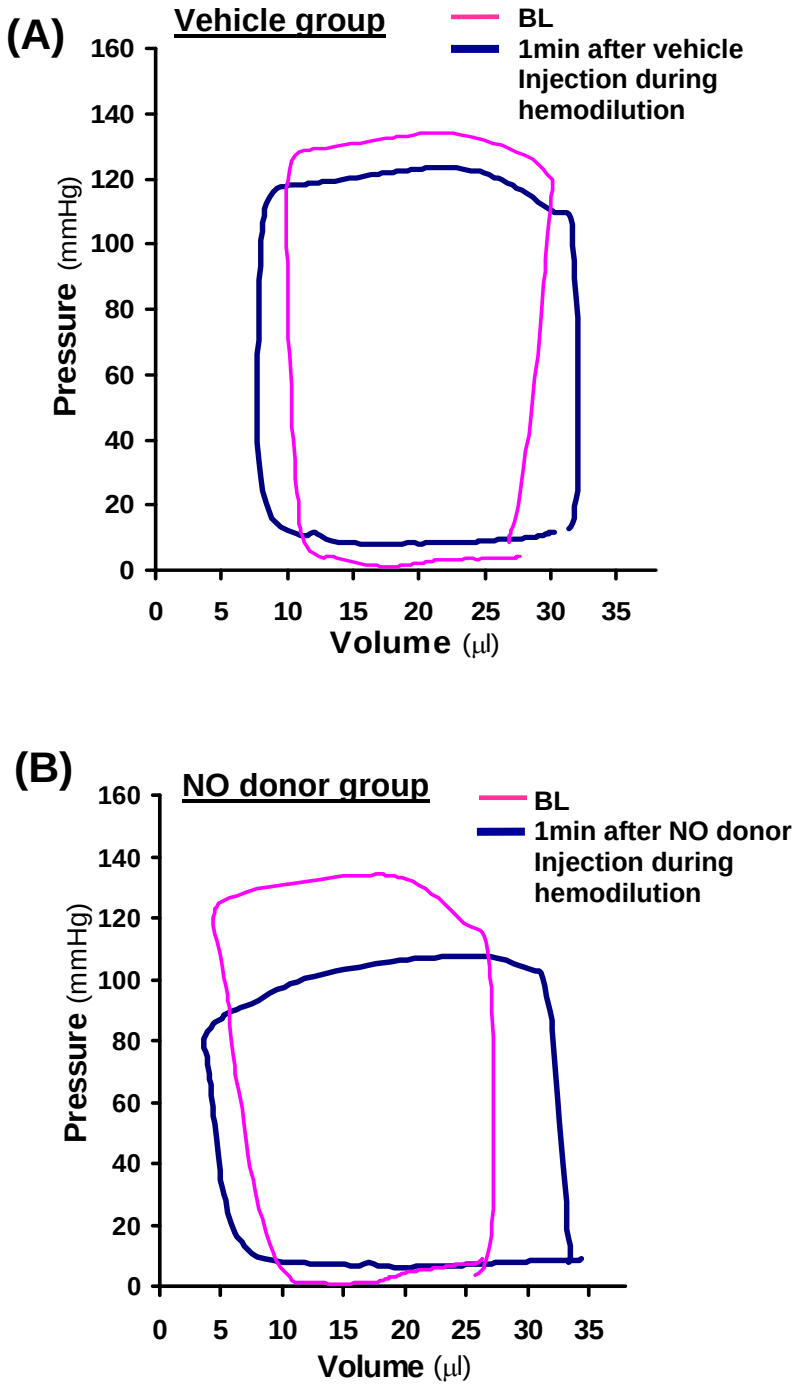

Figure 5.7: Pressure-volume loops at the baseline and 1 min after vehicle or NO donor infusion in (A) Vehicle group and (B) NO donor group.

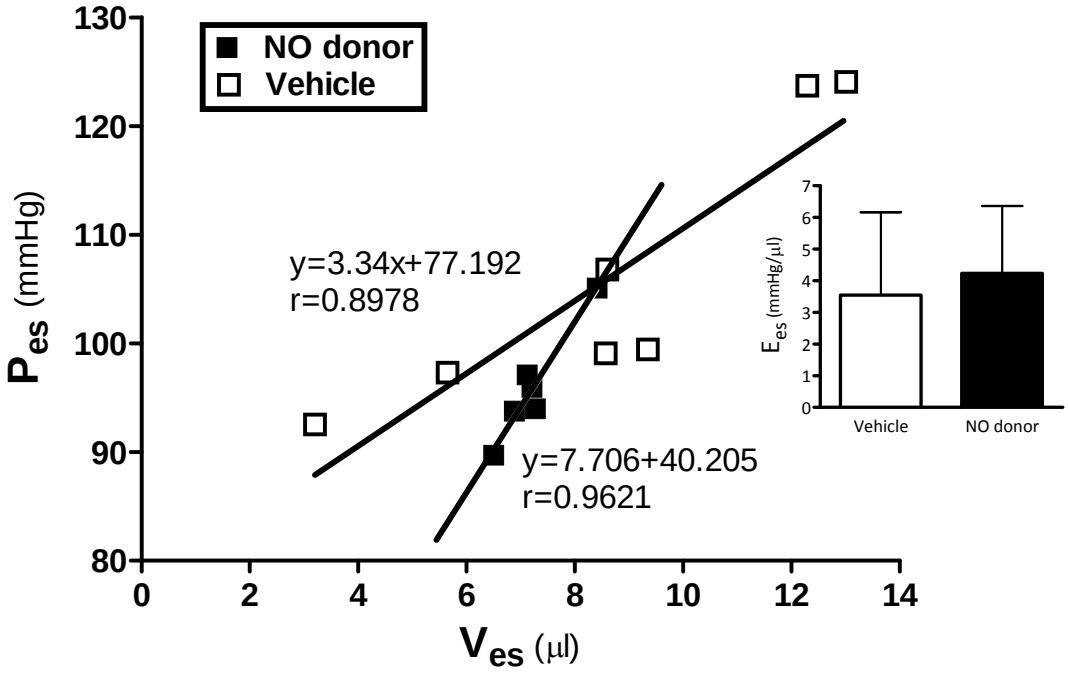

Figure 5.8: Relation between end-systolic pressure (P_{es}) and end-systolic volume (V_{es}) during 30 min after treatment. Solid lines are a linear regression for each experimental group (Vehicle: y=3.34x+77.192, r=0.8978; NO donor: y=7.706x+40.205, r=0.9621). Inset: overall slope values represented end-systolic elastance (E_{es}) as mean $\pm$ SD of 7 experiments in each group.

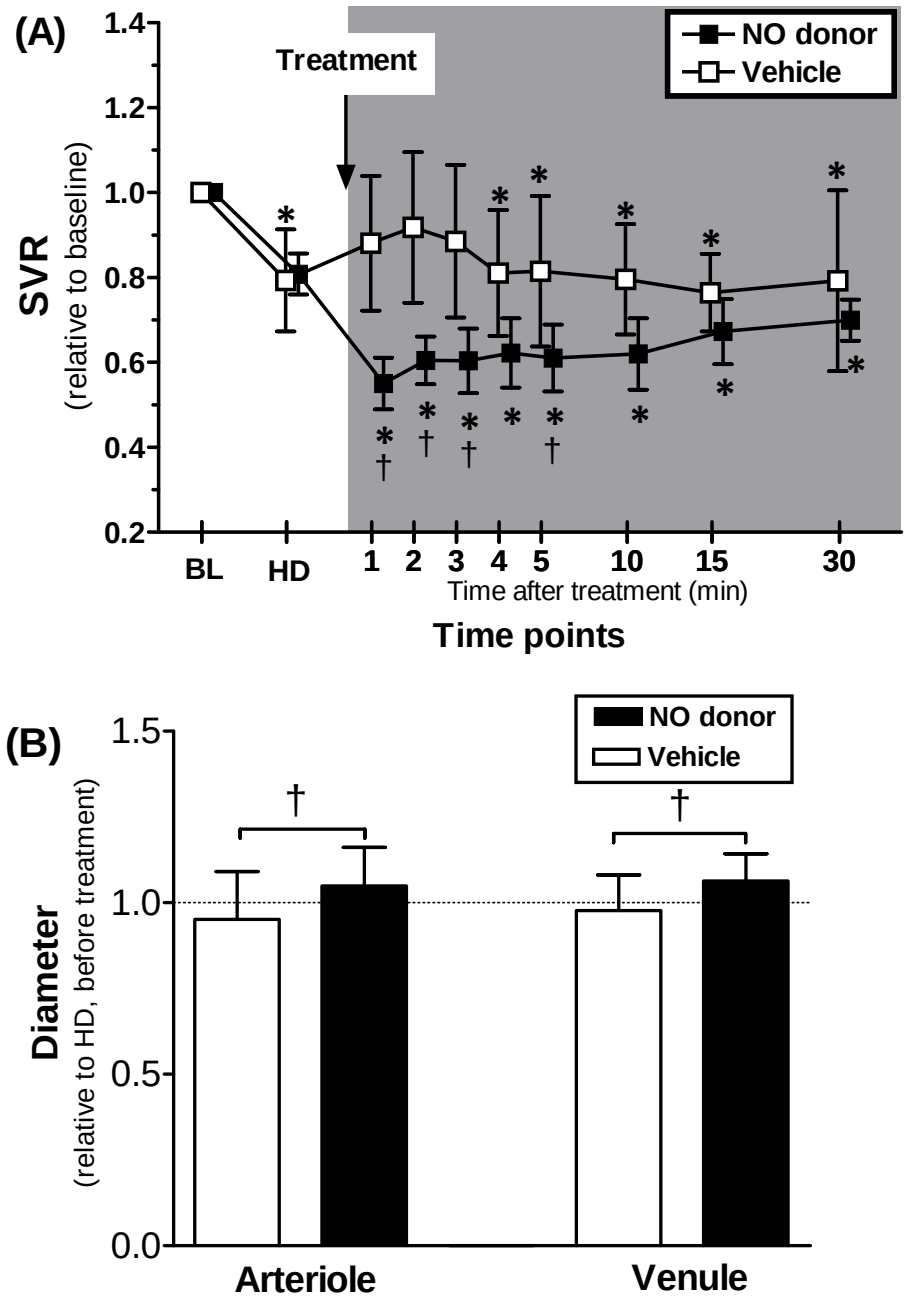

Figure 5.9: (A) Systemic vascular resistance (SVR) at baseline (BL), at 15 minutes after hemodilution (HD) and at 1, 2, 3, 4, 5, 10, 15 and 30 minutes after treatment. (B) Venular and arteriolar diameters relative to before treatment. Values are presented as means ± SD. * $p<0.05$ compared with BL. † $p<0.05$ between groups.

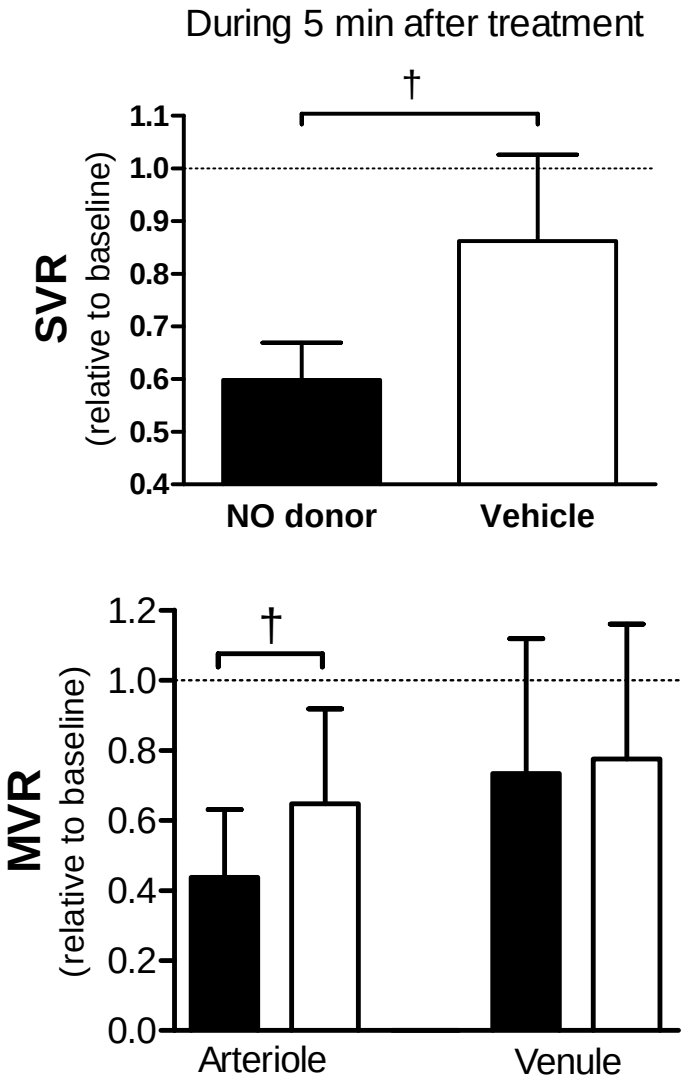

Figure 5.10: Systemic vascular resistance (SVR) and microvascular resistance (MVR) during 5 min after treatment relative to baseline. Values are presented as means ± SD. † $p<0.05$ between groups.

Discussions

The principal finding of this study is that increased vascular NO released by NO donor has beneficial effects on ventricular function by enhancing CO, SV and E_{es}, lowering SW/SV and SVR during an acute isovolemic hemodilution with LVPE. However, these beneficial effects are dose-dependent and not long-lasting. Our results support the concept that increased plasma viscosity induces shear stress-dependent NO release, leading to microvascular function improvement that implies a parallel improvement of cardiac function.

Previous studies in microcirculatory during acute hemodilution using the hamster window chamber model demonstrated the beneficial effects that elevated plasma viscosity with HVPE increased MAP and CO and improved microvascular function compared with LVPE but did not increase SVR [4, 5, 18]. Further evidence by Tsai et al. showed that elevated plasma viscosity increased perivascular NO concentration and microvascular perfusion in extreme hemodilution [33]. Thus, theses experimental studies elucidated the beneficial effects of NO in vascular bed. However, NO has bimodal effects, cardioprotective and cardiodepressive effects, on the heart depending on the amount of NO [3, 17, 29]. As we performed a dose-response testing, 25 mM of DETA NONOate resulted in animals' death, 1 mM of DETA NONOate did not significantly caused any changes in MAP but 10 mM of NO donor enhanced cardiac function. Unfortunately, the active concentration of NO in vivo is difficult to determine because the effect of NO is influenced by the scavenging activity of reactive oxygen species, hemoglobin content in red blood cells and myoglobin concentration in cardiomyocytes [19, 22]. In our study, the effects of NO were clearly observed during 10-15 min after

NO donor administration as similar to the previous finding with Pirsidomine by Wainwright and Martorana [36].

Vascular NO can affect HR and myocardial contractility as a result of NO released by coronary endothelial cells and cardiomyocytes. Generally, NO positively affects chronotropy. However, several studies in animals indicated that exogeneous NO can decrease baroreceptor activity and inhibit the chronotropic response [7, 20]. In our study, HR transiently increased in short term after NO donor administration as well as SV, leading to an increase of CO. Similarly, Hogan et al. showed that NO donor increased HR in anesthetized rabbit independent of the autonomic nervous system as a result of an stimulation of the hyperpolarization-activated inward pacemaker current [9]. Despite increased HR, the load-dependent systolic function index, dP/dt_{max}, was maintained as well as SW whereas P_{es} was lower relative to baseline after NO donor infusion, indicating that NO might not alter cardiac contractility. However, we found that E_{es} represented by a slope of the relationship between P_{es} and V_{es} was slightly higher in the group administered with NO donor, suggesting that NO had a positive inotropic effect. Some studies have reported that a positive inotropy caused by NO might be partially dependent on cGMP, which, when produced at low concentration, triggers cAMP production to activate protein kinase A (PKA), leading the opening of sarcolemmal voltage-operated and sarcoplasmic ryanodine receptors (Ry/R) Ca^{2+} channels [16, 31, 35]. NO itself can also open Ry/R, L-type Ca^{2+} channels and the calcium release complex by S-nitrosylation or oxidation [37].

In addition to the inotropic effect, NO affects the myocardial relaxation. Our result showed that NO significantly affected lusitropy as evidenced by a prominent

decrease of dP/dt_{min} and an early onset of relaxation phase shown in the PV loop. Furthermore, PV loop after NO donor administration shifted to the right relative to PV loop at the baseline, indicating increased left ventricular (LV) diastolic distensibility or preload. The increase of myocardial distensibility by NO effect probably relates to phosphorylation of troponin I and reduced calcium-independent diastolic crossbridge cycling. Despite increased preload due to NO donor, hemodilution also caused the increase in CO and end-diastolic volume to compensate the reduction in hematocrit and to maintain tissue oxygenation. Pinsky et al. reported their study in the beating rabbit heart that an increase in LV preload enhanced the myocardial NO production [28]. Therefore, in our study, not only NO was released from NO donor but also endogenous NO was produced in the NO donor group.

Comparing to the vehicle group, increased CO in the NO donor group was the result of in creased HR and SV responding to NO donor. Although SV increased, SW almost remained unchanged after NO donor infusion, indicating NO enhanced pumping performance without increasing oxygen consumption. Furthermore, the relationship between SW and CO showed a beneficial effect of NO on cardiac function because animals administered with NO donor had higher CO at the same work done by the heart compared with animals without NO donor. Similarly, the ratio of SW to SV in the NO donor group was lower compared to the vehicle group, implying less energy was used to pump out the same amount of blood. Other evidence showing the beneficial effect of NO was the reduction in systemic vascular resistance, leading to lower MAP and P_{es}. As the heart pumps against lower pressure or resistance, it partly implies that less energy is used to overcome afterload and is beneficial to the heart. Furthermore, our microvascular

observation in the window chamber model evidently showed that the reduction in systemic vascular resistance after NO donor infusion was a result of vasodilation. Knowing blood viscosity and vascular diameter, we demonstrated that the reduction of SVR after NO donor administration was in parallel with the decrease in MVR, especially in arterioles.

In conclusion, our findings demonstrate the beneficial effects of supplemented NO on cardiac function by increase in cardiac output, reduction of vascular resistance and lower in stroke work per ejected volume, in an acute hemodilution with low viscosity plasma expander. Our results further provide the evidences to support that endogenous NO induced by wall shear stress, due to increased plasma viscosity, has positive effects on cardiac function. Furthermore, it suggests that using NO donor has therapeutic potential for heart performance in anemic condition. However, NO donors have both positive and negative effects on cardiac function depending on the donor specie, dosage, release rate, pathological conditions and existence of endogenous NO.

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Chapter 6

The Contribution of Endothelial Glycocalyx Integrity in the Interaction with Viscogenic Plasma Expanders and its Effects on Cardiac and Vascular Function during Acute Isovolemic Hemodilution

Abstract

Endothelial glycocalyx involves in a mechanotransduction of flowing blood shear stress to endothelial cells. Modulation of plasma viscosity with viscogenic plasma expanders (PEs) in hemodilution influences the shear stress acted on the surface of endothelium and leads to different responses of endothelial cells. This study was designed to determine and quantify the interaction between endothelial glycocalyx and viscogenic PEs in vivo. An acute isovolemic hemodilution with 40% blood volume exchange was performed as a study protocol in pentobarbital-anesthetized hamsters. Endothelial glycocalyx was degraded by hyaluronidase, a specific enzyme for hyaluronic acid, via i.v. infusion. Dextran 2000 kDa (Dx2M), high viscosity PE (HVPE) with viscosity 6.2 cP, and polyethylene glycol conjugated with human serum albumin (PEG-HSA), low viscosity PE (LVPE) with viscosity 2.2 cP, were used in the blood exchange. Cardiac function was assessed by a miniaturized pressure-volume conductance catheter inserted to left ventricle. Experimental results showed that, at post-treatment state, mean arterial pressure decreased 8-11% from baseline in groups administered with hyaluronidase while it did not change in groups infused with vehicle. There was also markedly difference in stroke work (SW) and end-systolic pressure (P_{es}) between animals treated with hyaluronidase followed by hemodilution with PEG-HSA and animals hemodiluted with Dx2M ($p<0.05$). On the other hand, there was no significant difference

in SW and P_{es} between animals treated with vehicle followed by hemodilution with PEG-HSA and animals hemodiluted with Dx2M. It was also found that degradation of endothelial glycocalyx leads to fluid leakage to extravascular space as shown by a decrease of dry-to wet- weight ratio of excised heart, especially in animals administered with hyaluronidase and hemodilution with PEG-HSA. This study implicates the contribution of hyaluronic acid in the interaction with PEG-HSA during acute hemodilution and their interaction leads to the beneficial effects on cardiac and vascular function as similar as the results with Dx2M.

Keywords: endothelial glycocalyx; cardiac function; mechanotransduction; hemodilution; plasma viscosity; conductance catheter

Introduction

The glycocalyx, a thin hydrated gel-like layer lining on the vascular endothelium, has been identified as a barrier of macro molecules and adhesiveness of circulating blood cells i.e. white blood cells and platelets, providing an exclusion zone to these molecules and cells [4, 14, 24]. This thin layer also contributes to microvascular flow resistance and plays an important role in the regulation of vascular permeability [17-19, 22]. Recently, it has been proposed that the endothelial glycocalyx is a mechanosensor in transduction of fluid shear stress into intracellular cytoskeleton of endothelial cells, leading to the intracellular signaling activation and the production of vasoregulatory agents, mainly nitric oxide (NO) [10, 13, 20, 23].

Many experimental models, for example isolated vessel, cultured endothelial cells and isolated heart, have been performed to study the function of endothelial glycocalyx by enzymatic degradation of the glycocalyx components. Florian et al. removed heparan sulfate, glycocalyx-binding glycosaminoglycan, with heparinase III from the cultured endothelial cells and found that, under steady and oscillatory shear stress, NO_x (NO_2^- and NO_3^-) production was substantially inhibited with heparinase treatment [8]. Furthermore, Pahakis and coworkers used enzymes that selectively degraded glycocalyx components from the surface of bovine aortic endothelial cells to study the role of endothelial glycocalyx components in mechanotransduction of fluid shear stress [16]. They found that the depletion of heparan sulfate (HS), hyaluronic acid (HA) and sialic acid (SA), but not chondroitin sulfate (CS), inhibited shear-induced NO production. Mochizaki et al. reported that HA within glycocalyx plays a crucial role in sensing and transducing shear force of flowing blood that triggers endothelial-derived NO production in isolated canine

femoral arteries [13]. Conclusively, these studies have revealed the important role of glycocalyx in NO release induced by shear stress as a mechanotransducer. Microhemodynamics following glycocalyx degradation with hyaluronidase has been recently studied in animal model by Cabrales and coworkers [6]. Using hamster window chamber model, they demonstrated that the decrease in functional capillary density coupled with the increase in hematocrit (Hct) in the remaining capillary, suggesting that the reduction of glycocalyx layer results in increased capillary Hct and a significant redistribution of red blood cell flow in the capillary network. However, the role of glycocalyx in physiological and pathological conditions in vivo has partially been elucidated.

Several in vivo studies have demonstrated that changing blood and plasma viscosity affects vascular wall shear stress and vascular NO production. Tsai et al. performed their experiment in a hamster window chamber model and reported that elevated plasma viscosity in extreme hemodilution increased arteriolar- and venular- wall shear stresses, microvascular perfusion and perivascular NO production [21]. Furthermore, their study showed significantly increased aortic endothelial NO synthase (eNOS) protein expression when animals were hemodiluted with HVPE compared with LVPE. Interestingly, recent study by Cabrales and colleagues demonstrated that low viscosity PE such as polyethylene glycol conjugated with albumin (PEG-Alb) significantly increased cardiac index and enhanced microvascular perfusion and vasodilation better than dextran 70 kDa (Dx70), a higher viscosity PE.[5] Their study may imply that PEG-Alb caused shear stress-induced NO higher than Dx70, leading to a greater vasodilation. Moreover, Jacob et al showed that the endothelial glycocalyx

prefers albumin for releasing shear stress-induced NO to mediate coronary dilation [10]. By these studies, it evidently suggests that PEG-Alb may differently interact with endothelial glycocalyx in a different manner compared to Dx70.

In the present study, we hypothesized that PEG conjugated with human serum albumin (PEG-HSA) interacts with endothelial glycocalyx, leading to the beneficial effect on cardiac function as elucidated in an improvement of microvascular perfusion observed in microcirculation study. To test this hypothesis, we administered Streptomyces hyaluronidase to degrade hyaluronic acid and assessed left ventricular function using a miniaturized pressure-volume conductance catheter in an anesthetized hamster under acute isovolemic hemodilution using PEG-HSA and HVPE.

Methods and Materials

Animal preparation for cardiac function measurement

Studies were performed in anesthetized male Golden Syrian hamsters (Charles River Laboratories; Boston, MA) weighing 60-65g. Animal handling and care followed the NIH Guide for Care and Use of Laboratory Animals. The experimental protocol was approved by the local animal care committee. Surgery was performed following i.p. injection of sodium pentobarbital (50mg/kg). The left jugular vein was catheterized to allow fluid infusion and left femoral artery was cannulated for blood pressure monitoring and blood withdrawal and sampling. Furthermore, animal was performed tracheotomy and cannulated with a polyethylene-90 tube to facilitate spontaneous breathing. Animal was placed in the supine position on the heating pad to maintain the body temperature at 36-37 ^{o}C. During the experiment, if animals response to a toe pinching, a small bolus of

sodium pentobarbital (10-15mg/kg, i.p.) will be given.

Animal preparation for a skin fold window chamber

The hamster skin fold window chamber model is widely used for microvascular studies in the unanesthetized state, and the complete surgical technique for the preparation has previously been described in detail [21]. The animal was allowed at least 2 days for recovery; its chamber was then assessed under the microscope for any signs of edema, bleeding, or unusual neovascularization. Arterial and venous catheters (polyethylene-50) were implanted in the carotid artery and jugular vein, respectively. Catheters were tunneled under the skin and exteriorized at the dorsal side of the neck.

Inclusion criteria

Animals under anesthesia were suitable for the experiments if 1) animals had no bleeding and 2) systemic parameters were within normal range, namely, mean blood arterial pressure (MAP) above 80 mmHg, heart rate (HR) above 320 beats/minute and systemic hematocrit (Hct) above 45%.

Systemic parameters

The MAP was monitored continuously (MP150, Biopac System Inc.; Santa Barbara, CA), except during blood exchange. The Hct was determined from centrifuged arterial blood samples taken in heparinized capillary tubes (Readacrit Centrifuge, Clay Adams, Division of Becton Dickinson; Parsippany, NJ).

Isovolemic hemodilution protocols

Anesthetized hamsters were exchanged by 40% of estimated total blood volume (BV) with test plasma expanders, lowering systemic Hct by 45%. Total BV was estimated as 7% of body weight. Animals were treated with hyaluronidase or vehicle by

a bolus injection (0.15 ml i.v.) after the baseline and hemodilution was performed at 45 min after hyaluronidase or vehicle administration. Test plasma expander was infused into the left jugular vein catheter at a rate of 0.1 ml/min with simultaneous blood withdrawal at the same rate from the left femoral artery catheter by using a dual syringe pump (33 syringe pump, Harvard Apparatus, Holliston, MA). Blood sample was collected at the end of experiment for blood conductance measurement. Animals were monitored for 60min after hemodilution. Systemic parameters (MAP and Hct) were recorded and analyzed at baseline and after hemodilution. Figure 6.1 illustrates the experimental protocols.

Biophysical properties

Viscosity was measured at a shear rate $160s^{-1}$ (Brookfield Engineering Laboratories; Middleboro, MA). Colloid osmotic pressure (COP) of PE was determined using a membrane colloid osmometer (model 420, Wescor; Logan, UT).

Cardiac function measurement

Closed chest method was performed to assess cardiac function in this study [15]. The right common carotid artery was exposed allowing a 1.4F pressure-volume conductance catheter (PV catheter; SPR-839, Millar Instruments, TX) to be inserted. The PV catheter was advanced passing through the aortic valve into the left ventricle. At the baseline and the end of experiment, a bolus of 15% hypertonic saline (10 μl) was intravenously injected to determine the parallel volume (V_p) [2]. The pressure and volume signals were instantaneously digitized and acquired (MPVS300, Millar Instruments; Houston, TX and PowerLab 8/30, ADInstruments; Colorado Springs, CO).

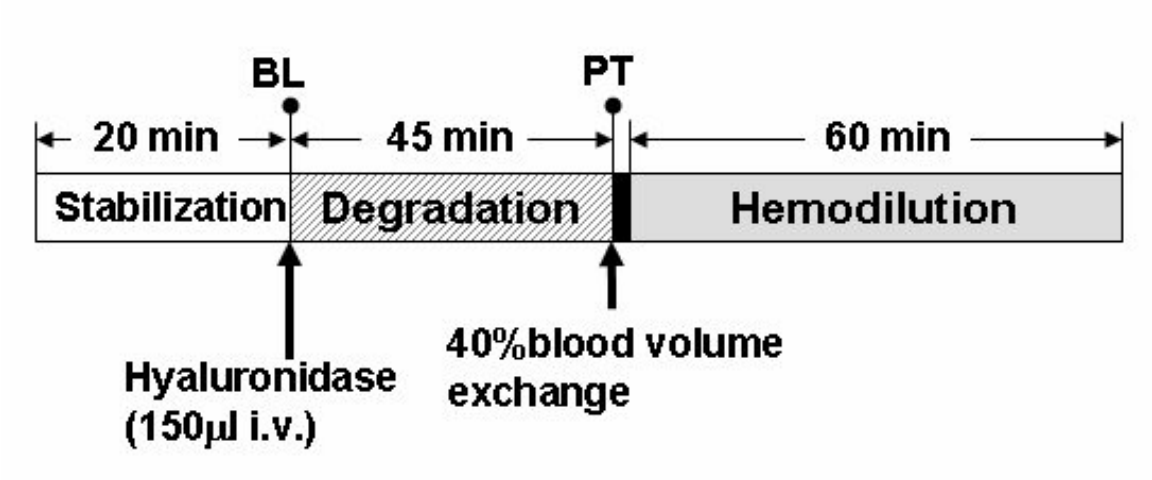

Figure 6.1: Schematic diagram of an acute isovolemic hemodilution protocol. A bolus of hyaluronidase was administered (150 µl i.v.) after baseline measurement. BL, baseline; PT, post-treatment.

Cardiac function data were analyzed with PVAN software (version3.6, Millar Instruments, TX). The values of studied cardiac function indices were averaged from selected 8-12 cardiac cycles at each time point.

Vascular resistance

Systemic vascular resistance (SVR), the resistance to flow in the peripheral circulation, was mathematically calculated from the measured values as SVR =MAP/CO, where MAP is the mean arterial pressure and CO is cardiac output.

Vascular diameter measurement

Anesthetized animals were placed in a restraining tube with a longitudinal slit from which window chamber protruded and then were fixed to the microscopic stage of a transillumination intravital microscope (BX51WI; Olympus, New Hyde Park, NY). The tissue image was projected onto a charge-coupled device camera (COHU 4815), connected to a videocassette recorder (AG-7355, JVC) and viewed on a monitor. Measurements were carried out using a ×20 water immersion objective (UMPlanFI 20×/0.50, Olympus). A video image-shearing method was used to measure the vessel diameter at the baseline and 30- and 60- min after hemodilution [9].

Functional capillary density

Functional capillary density (FCD) is defined as the number of capillary segments with passing of at least a single red blood cell in a 60-s period per microscopic observation field. Detailed mappings were prepared of the chamber vasculature so that the same vessels studied at baseline could be followed throughout the experiment. This parameter is evaluated in 5-6 successive microscopic fields. The field is chosen by a

distinctive anatomic landmark, i.e., large microvascular bifurcation, to easily and quickly reestablish the same observation field at each observation time point.

Enzyme and vehicle treatment

Streptomyces hyaluronidase (Sigma Chemical, St. Louis, MO) was prepared with dose of 100 units in saline, equivalent to at least 40 U/ml plasma as previously described by Cabrales et al. that this dose of 100 units of hyaluronidase produced the distinguishable effect in microvascular study [6]. In this study, 0.9% saline was given as a vehicle. The hyaluronidase or vehicle was given in a bolus doses (150 μl) via jugular vein catheter after the baseline.

Test solutions

Two solutions were as follows: 1) polyethylene glycol (PEG) conjugated with 4g/dl human serum albumin (Alb; Sigma-Aldrich, St. Louis, MO) and 2) 6% Dextran 2000 kDa (Pharmacosmos, Holbaek, Denmark) in 0.9% sodium chloride mixed with 10% human serum albumin. The process for PEGylation and conjugation of PEG-HSA was previously described by Martini et al.[12]. Table 6.1 lists the physical properties of these test solutions.

Table 6.1: Physical properties of the solutions

Solution	Viscosity (cP)	COP (mmHg)
PEG-HSA	2.2	58
Dx2M	6.2	43

Viscosity was measured at shear rate of 160 s^{-1} at 37^{o}C.
Values are means.

Experimental groups

Animals were randomly divided into four groups. A first group was treated with hyaluronidase followed by hemodilution with PEG-HSA (group labeled Enz-PEG). A second group was treated with hyaluronidase followed by hemodilution with Dextran 2000 kDa (group labeled Enz-Dx2M). A third group was administered with 0.9% saline followed by hemodilution with PEG-HSA (group labeled with Vehicle-PEG). A forth group was administered with 0.9% saline followed by hemodilution with Dx2M (group labeled with Vehicle-Dx2M).

Estimation of left ventricular blood volume

Left ventricular blood volume was measured continuously in conductance units (RVU; relative volume unit) and converted to actual blood volume (μl) at the end of the experiment. The blood conductance at the baseline was estimated from baseline hematocrit, using the pooling data of blood conductance and hematocrit relationship (see Appendix II).

Dry- to wet- heart weight measurement

The effect of hyaluronidase on fluid leakage to extravascular tissue was assessed in the whole heart. At the completion of experiment, animal was sacrificed, the chest was opened and the heart was excised, weighted, dried with a microwave oven at 65 WA power-level for 3 min and weighed again to quantify the fluid content in the heart. Dry- to wet- weight ratio was then calculated.

Statistical analysis

Results are presented as mean ± standard deviation (SD) unless otherwise noted. All data are presented as absolute values and ratios relative to baseline values. Data

between interested time points in a same group were analyzed using analysis of variance for repeated measurement (ANOVA) and followed by post hoc analyses with the Dunnett's multiple comparison tests. An unpaired t-test was performed to compare data between groups. All statistics were calculated using GraphPad Prism 4.01 (GraphPad Software, San Diego, CA). Results were considered statistically significant if $p<0.05$.

Results

Animals were randomly assigned to the experimental groups: Enz-PEG (n=7, 63±2 g), Vehicle-PEG (n=7, 63±1 g), Enz-Dx2M (n=7, 64±2 g) and Vehicle-Dx2M (n=7, 63±2 g). Five animals in each group were used for cardiac function study and two animals in each group were used for vascular diameter and FCD measurement in window chamber. Systemic Hct similarly dropped from 51$\pm$3 % at the baseline to 27$\pm$2 % in all groups after hemodilution.

Figure 6.2 presents MAP and HR at baseline, post-treatment, 15-, 30- and 60-min after hemodilution. MAP in groups treated with hyaluronidase significantly decreased relative to baseline and groups treated with vehicle at post-treatment state ($p<0.05$). Animals treated with hyaluronidase followed by hemodilution with Dx2M had significantly higher MAP than animals treated with hyaluronidase followed by hemodilution with PEG-HSA during hemodilution ($p<0.05$). HR in the groups hemodiluted with Dx2M markedly dropped relative to baseline and groups hemodiluted with PEG-HSA ($p<0.05$). However, HR in the groups hemodiluted with Dx2M slightly increased after 15-min post hemodilution while it gradually decreased in the groups hemodiluted with PEG-HSA.

During hemodilution, cardiac output (CO) in animals treated with hyaluronidase followed by hemodilution with Dx2M and animals treated with vehicle and hemodiluted with PEG-HSA was slightly higher than that in animals treated with hyaluronidase followed by hemodilution with PEG-HSA. CO in animals treated with vehicle and hemodiluted with Dx2M gradually increased over the period of experiment during hemodilution. However, there was no significant difference between groups in CO. Figure 6.3A shows stroke volume (SV) of all experimental groups at post-treatment state and 60 min during hemodilution. Only animals hemodiluted with Dx2M had a significant increase in SV relative to baseline during first 30 minutes of hemodilution ($p<0.05$) and their SV was slightly higher compared with animals hemodiluted with PEG-HSA. No significant difference in stroke work (SW) between groups was observed at the post-treatment state as shown in Figure 6.3B. On the other hand, at 15 min of hemodilution, SW in a group treated with hyaluronidase followed by hemodilution with Dx2M was remarkably higher than a group infused with hyaluronidase and hemodiluted with PEG-HSA ($p<0.05$). Figure 6.3C presents the ratio between SW to SV, representing the external work that the heart pumps blood out. At 15 min of hemodilution, the SW/SV ratio in animals treated with vehicle and hemodiluted with PEG-HSA was higher than animals treated with hyaluronidase followed by hemodilution with PEG-HSA and similar to animals infused with hyaluronidase followed by hemodilution with Dx2M ($p<0.05$).

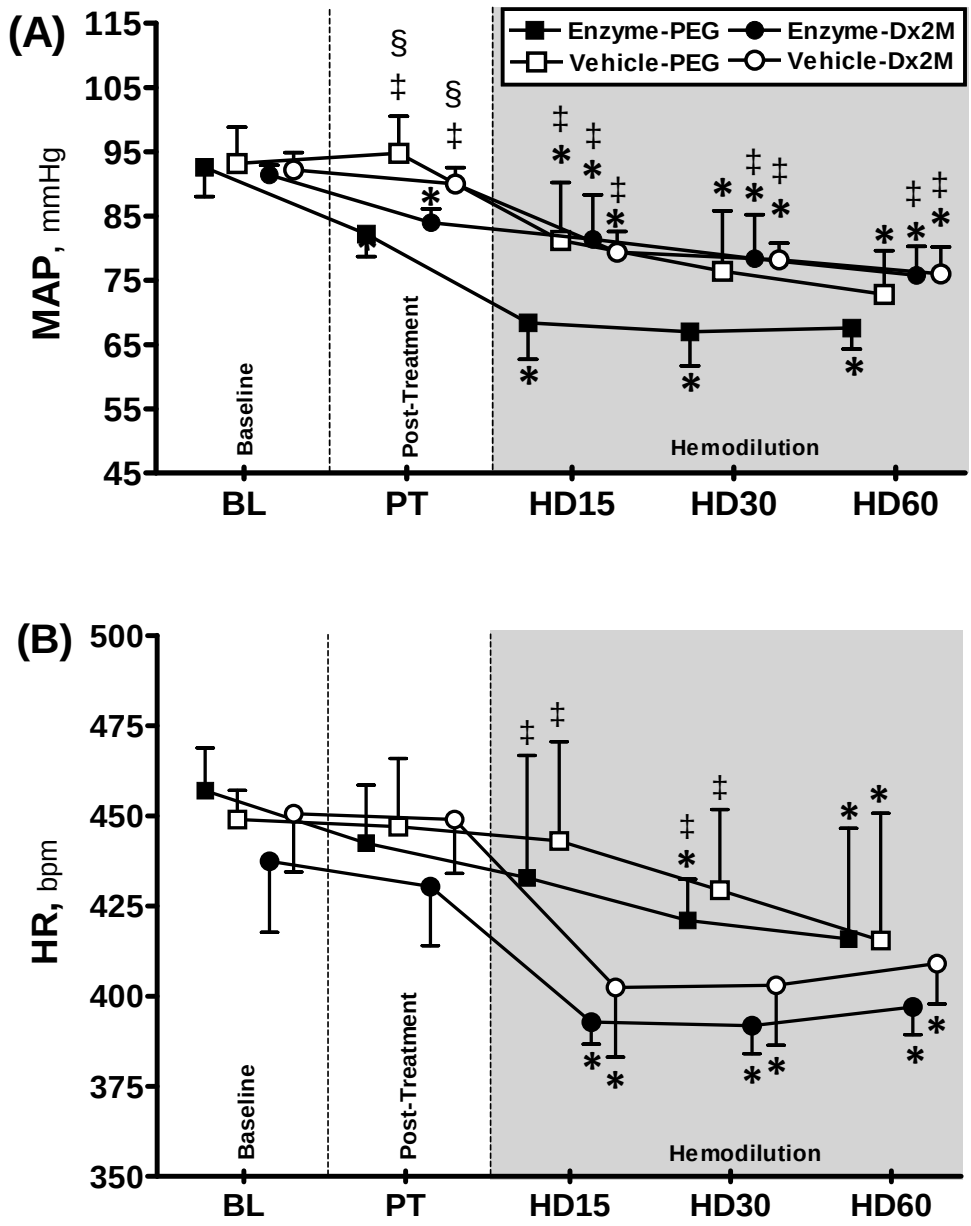

Figure 6.2: (A) Mean arterial pressure (MAP) and (B) Heart rate (HR) measured at baseline (BL), post-treatment (PT) and 15, 30 and 60 minutes after hemodilution (HD15, HD30, HD60). Values are presented as means ± SD. * $p<0.05$ compared with baseline in a same group. ‡ $p<0.05$ compared with the Enzyme-PEG group. § $p<0.05$ compared with the Enzyme-Dx2M group.

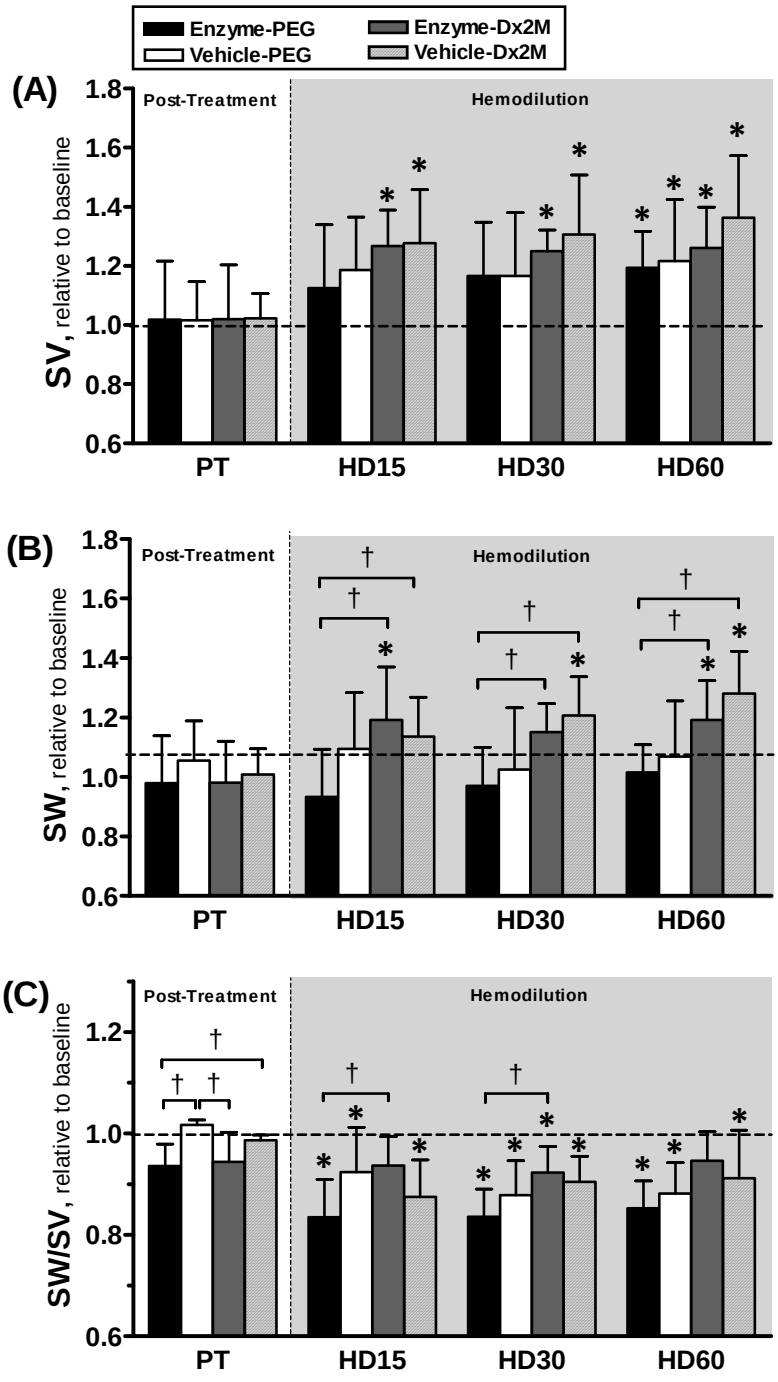

Figure 6.3: (A) Stroke volume (SV), (B) Stroke work (SW) and (C) Stroke work per stroke volume (SW/SV) at post-treatment (PT) and 15, 30 and 60 minutes after hemodilution (HD15, HD30, HD60). Broken line represents the values at baseline. Values are presented as means ± SD. * $p<0.05$ compared with baseline. † $p<0.05$ compared between groups.

End-systolic pressure (P_{es}) in a group treated with hyaluronidase followed by hemodilution with PEG-HSA significantly dropped from the baseline and was obviously lower than that in the other groups as displayed in Figure 6.4A. In contrast, end-diastolic pressure (P_{ed}) in animals administered with hyaluronidase and hemodiluted with PEG-HSA was higher than that in animals hemodiluted with Dx2M and nearly similar to P_{ed} in animals treated with vehicle and hemodiluted with PEG-HSA as shown in Figure 6.4B. At the post-treatment state, the maximum rate of pressure change (dP/dt_{max}) reduced by 10-12% from the baseline in groups treated with PEG-HSA whereas it maintained at the baseline in groups infused with vehicle (Figure 6.5A). At 30 and 60 min during hemodilution, dP/dt_{max} in animals hemodiluted with Dx2M (84-90% of baseline) was higher compared to that in animals hemodiluted with PEG-HSA (69-75% of baseline). Figure 6.5B shows the minimum rate of pressure change (dP/dt_{min}). Animals treated with hyaluronidase demonstrated, at post-treatment state, a decrease of dP/dt_{min} while animals without hyaluronidase had dP/dt_{min} at the baseline level. Furthermore, at 60 min of hemodilution, dP/dt_{min} in animals treated without hyaluronidase was still higher than that in animals treated with hyaluronidase.

Hemodilution significantly reduced systemic vascular resistance (SVR) in all groups but no significant difference between groups was found as shown in Figure 6.6. No significant difference between groups was observed in arteriolar and venular diameters over the period of experiment as presented in Figure 6.7A and B, respectively. Figure 6.7C shows functional capillary density (FCD). At the post-treatment state, FCD in groups treated with vehicle maintained at the baseline level while it decreased in

groups infused with hyaluronidase ($p<0.05$). Moreover, during hemodilution, FCD in a group infused with hyaluronidase was lowest among all experimental groups ($p<0.05$).

Figure 6.8 shows the dry to wet weight ratio of excised heart after the end of experiment. This ratio in a group treated with hyaluronidase followed by hemodilution with PEG-HSA was promisingly lower compared to other groups ($p<0.05$).

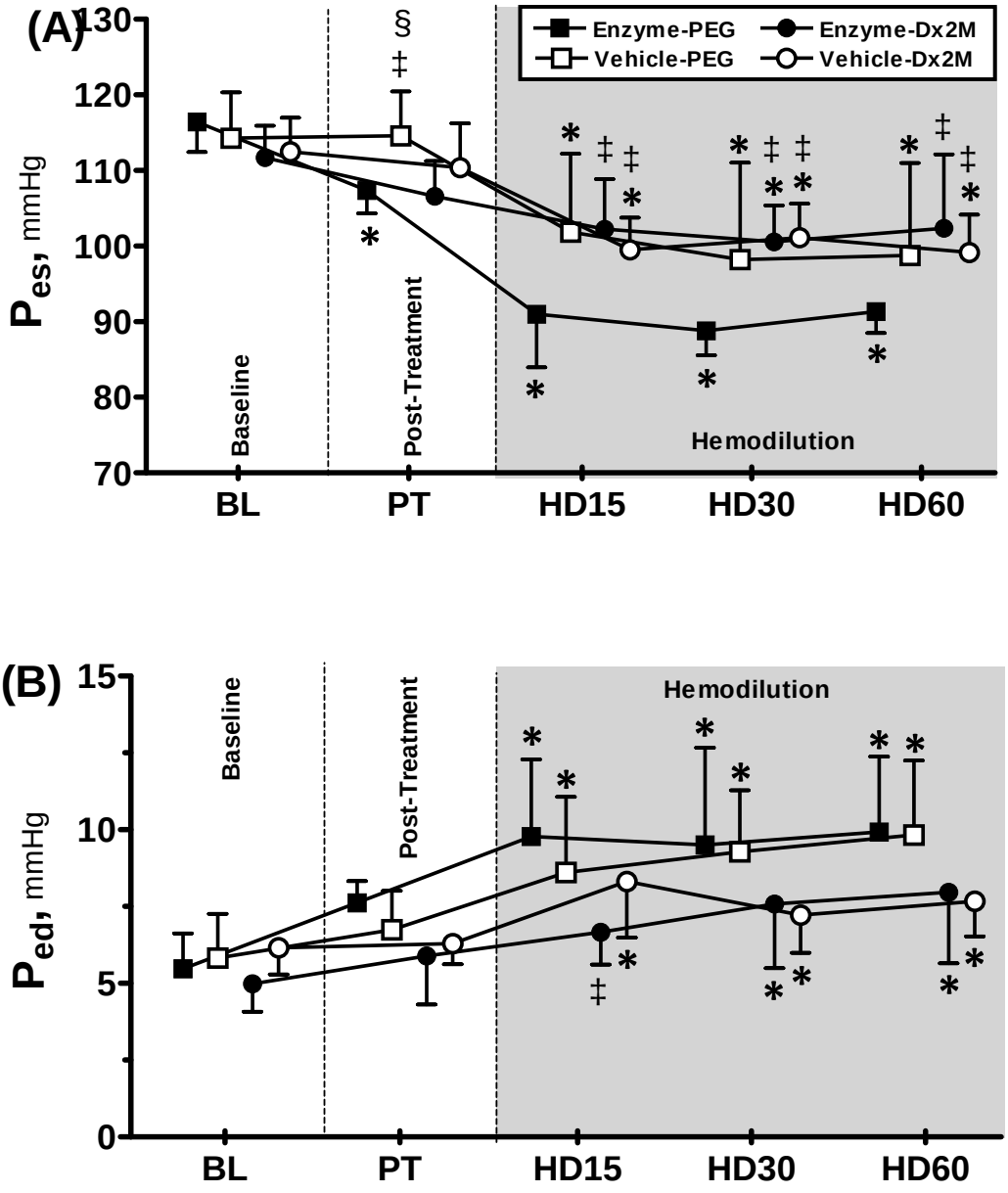

Figure 6.4: (A) End-systolic pressure (P_{es}) and (B) End-diastolic pressure (P_{ed}) at baseline (BL), post-treatment (PT) and 15, 30 and 60 min after hemodilution (HD15, HD30, HD60). Values are presented as means ± SD. * $p<0.05$ compared with baseline within a same group. ‡ $p<0.05$ compared with the Enzyme-PEG group. § $p<0.05$ compared with the Enzyme-Dx2M group.

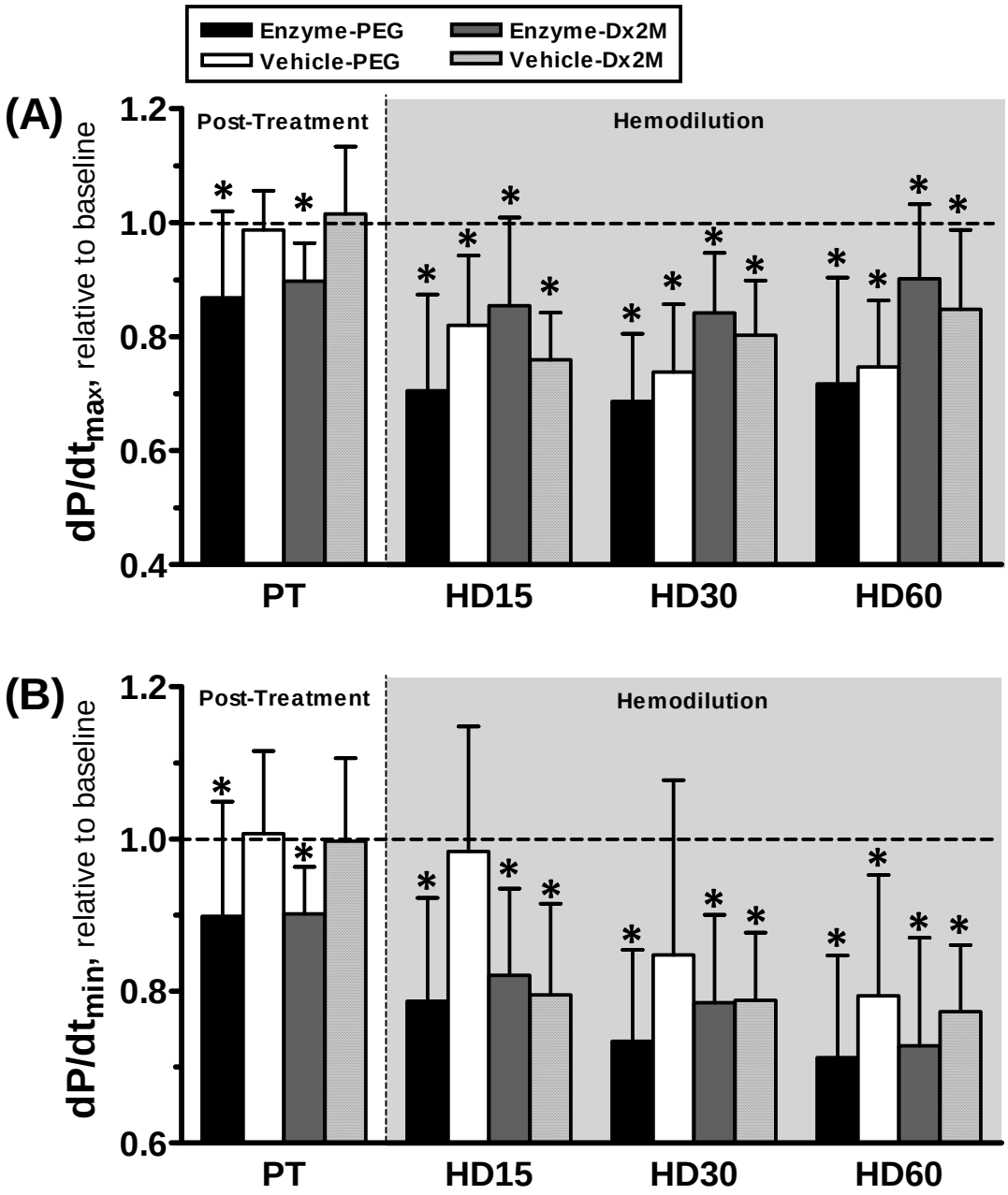

Figure 6.5: (A) Maximum rate of pressure change (dP/dt_{max}) and (B) Minimum rate of pressure change (dP/dt_{min}) at baseline (BL), post-treatment (PT) and 15, 30 and 60 min after hemodilution (HD15, HD30, HD60). Broken line represents the values at baseline. Values are presented as means ± SD. * $p<0.05$ compared with baseline within a same group.

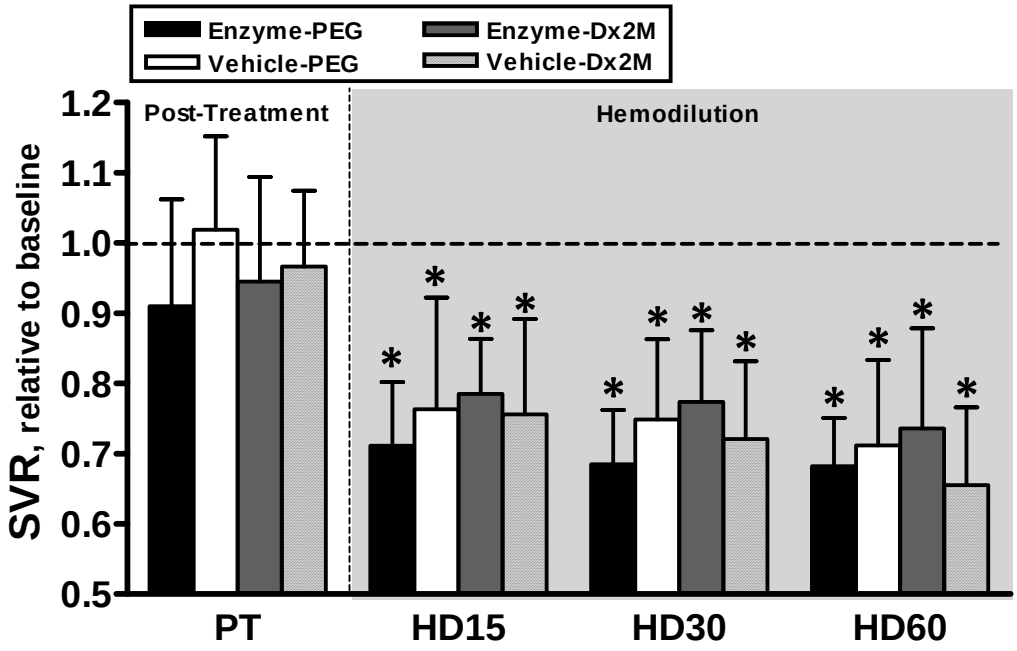

Figure 6.6: Systemic vascular resistance (SVR) at post-treatment (PT) and 15, 30 and 60 min after hemodilution (HD15, HD30, HD60). Broken line represents the values at baseline. Values are presented as means ± SD. * $p<0.05$ compared with baseline within a same group.

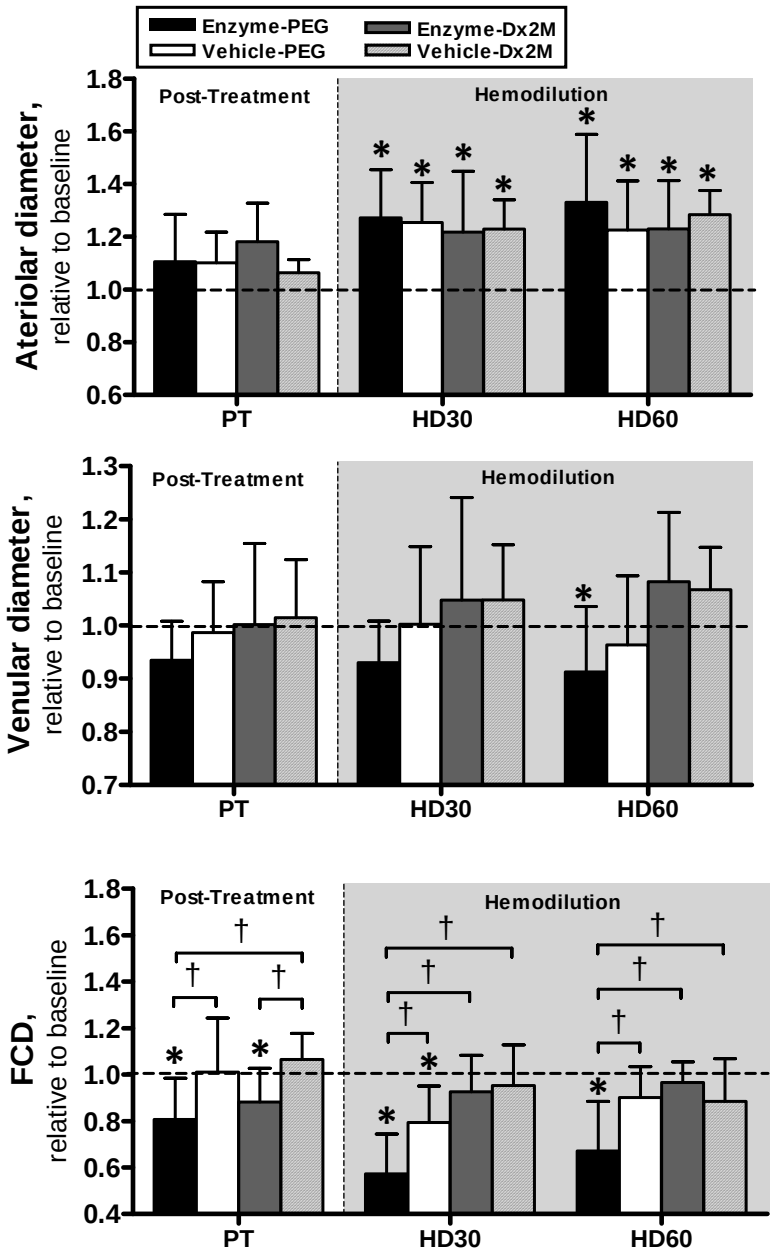

Figure 6.7: Mircovascular parameters from an awake hamster window chamber model. (A) Arteriolar diameter, (B) Venular diameter and (C) Functional capillary density (FCD) at post-treatment (PT) and 30 and 60 min after hemodilution (HD30, HD60). Broken line represents the values at baseline. Values are presented as means ± SD. * $p<0.05$ compared with baseline within a same group. † $p<0.05$ compared between groups.

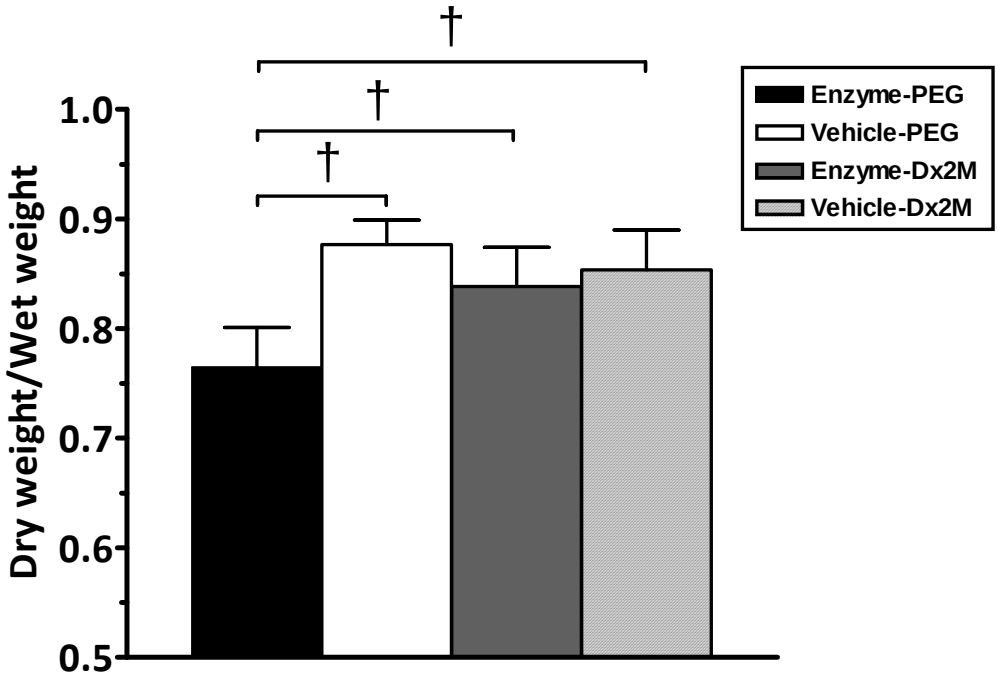

Figure 6.8: Dry- to wet- weight ratio of excised heart at the end of experiment. † $p<0.05$ compared between groups.

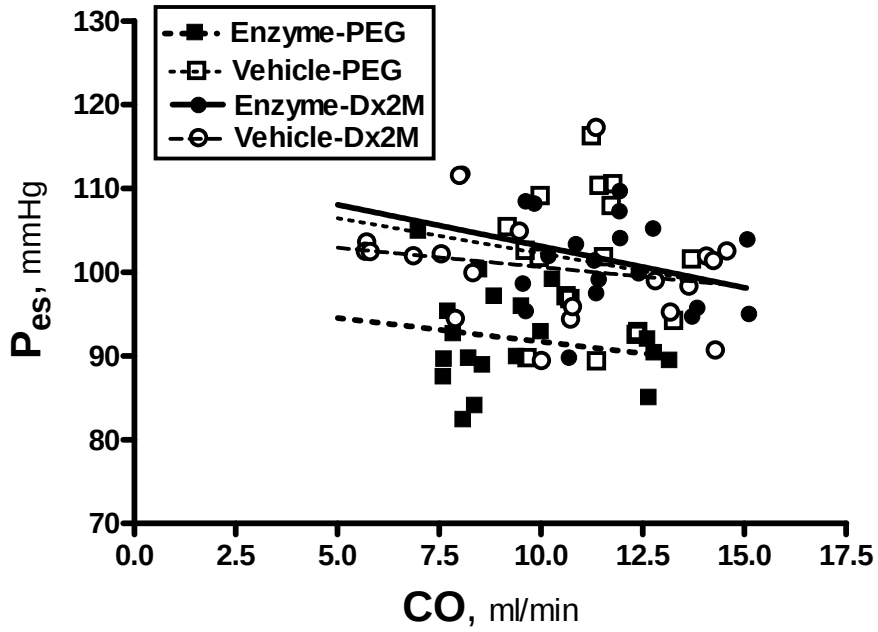

Figure 6.9: Relation between end-systolic pressure (P_{es}) and cardiac output (CO) during 60 minutes of hemodilution. Lines are a linear regression for each experimental group (Enzyme-PEG: y=-0.57x+97.35, r=0.19; Vehicle-PEG: y=-0.84x+110.64, r=0.14; Enzyme-Dx2M: y=-0.99x+113, r=0.32; Vehicle-Dx2M: y=-0.46x+105.23, r=0.22).

Discussions

The principal finding of this study is that PEG-HSA interacts with endothelial glycocalyx via hyaluronic acid and they contribute to endothelial mechanotransduction, leading to beneficial effects on cardiac and vascular function in an acute hemodilution. Hyaluronic acid does not play a role in an interaction with Dx2M (HVPE). Although PEG-HSA has lower viscosity and different mechanism in the endothelial mechanotransduction compared to Dx2M but they have similar positive effects on cardiac and vascular function, including increased load-dependent indices such as SV and CO, decreased SVR and sustained SW/SV ratio. Furthermore, myocardial edema as a result of glycocalyx degradation contributes in a deterioration of pumping performance of the heart as observed in animals administered with hyaluronidase followed by hemodilution with PEG-HSA.

Vascular endothelial glycocalyx degradation using Streptomyces hyaluronidase, a selective enzyme of hyaluronic acid, increased vascular permeability of larger molecules as reported by Cabrales et al.[6], leading to vascular extravasation and tissue edema as shown in our result by a decreased dry- to wet- weight ratio of excised heart. We further found that MAP, P_{es} and dP/dt_{max} markedly decreased after hyaluronidase administration compared to baseline and animals without hyaluronidase administration. These findings imply that endothelial glycocalyx degradation, in part, negatively affects systolic function of the heart. The effects of glycocalyx degradation on cardiac function may be resulted in not only a systemic circulatory system but also coronary circulation and endocardial endothelial cells. In addition, microvascular perfusion evaluated by FCD in awake model was attenuated in animals treated with hyaluronidase as showed by a significant reduction

of FCD relative to baseline and animals without hyaluronidase treatment. Therefore, the reduction of FCD may be observed in anesthetized animals treated with hyaluronidase and possibly correlate to attenuation in heart perfusion, leading to the deteriorated cardiac pumping performance. On the other hand, Evora et al. suggested that exogenous hyaluronidase induces release of nitric oxide (NO) from coronary endothelium in the excised coronary artery by binding to hyaluronidase receptors on endothelium, possibly leading to a protective effect on coronary reperfusion.[7] However, the acute endothelial glycocalyx degradation in an intact physiological condition, a post-treatment state in our study, did not demonstrate a significant change in other cardiac function indices such as SV, CO and SW. Recently, Brands et al. reported that acute attenuation of glycocalyx using hyaluronidase increases coronary blood volume and coronary conductance.[3] Their findings, in part, support our result that calculated SVR decreased after hyaluronidase infusion, implying a similar effect may be observed in coronary circulation.

Early studies proposed that endothelial glycocalyx may play an important role as a shear stress mechanotransducer and affect vascular function such as contractile function.[1, 8, 26] Therefore, the degradation of endothelial glycocalyx combined with changing blood or plasma viscosity may affect vascular function and circulatory system. Our study with hyaluronidase administration in an acute hemodilution is an approach to determine and quantify the interaction between endothelial glycocalyx and plasma expanders in vivo. We found that hyaluronic acid plays a role in the interaction with PEG-HSA, not with HVPE, dextran 2000 kDa (Dx2M), as shown by higher values of cardiac function indices and MAP in animals treated with hyaluronidase and hemodiluted

with Dx2M. The plot between CO and P_{es} represents a pump function curve as shown in Figure 6.9. The degradation of endothelial glycocalyx shifts the pump curve down during hemodilution with PEG-HSA but it does not change the pump curve when hemodilution with Dx2M. We interpreted that, at the same CO, lower P_{es} after hyaluronidase administration followed by hemodilution with PEG-HSA indicates lower pressure gradient leading to lower peripheral perfusion pressure. Furthermore, FCD in awake animals treated with hyaluronidase and hemodiluted with Dx2M was higher than that in animals treated with hyaluronidase and hemodiluted with PEG-HSA, emphasizing the role of hyaluronic acid in the interaction with PEG-HSA. Changing blood and plasma viscosity affects shear stress acted on the endothelial cells. Cabrales and co-workers calculated wall shear rate (WSR) and wall shear stress (WSS) in arterioles and venules of different plasma expanders and showed that PEG-HSA caused a similar order of magnitude of WSR but lower WSS compared to HVPEs.[5] Recent study by Jacob et al. has indicated that albumin induces greater endothelial shear stress than hydroxylethyl starch (6% HES; 200/0.5 or 450/0.7), although it has lower viscosity.[10] They also found that coronary blood flow decreased after glycocalyx degradation with heparinase, despite infusion with albumin. Their study evidently provides the advantage of albumin to endothelial glycocalyx. In contrast, arteriolar and venular wall shear stress calculated by Cabrales et al. for 5% human serum albumin (HSA) was lower than that for 10% HES 200.[5] Thus, using PEG-HSA in our study, not only albumin but also PEG plays a major role in the interaction with endothelial glycocalyx.

Our study showed that there were no significant differences in cardiac function indices and SVR between animals treated with and without hyaluronidase but they were

hemodiluted with Dx2M (HVPE). Therefore, it is evidence that degradation of hyaluronic acid did not affect cardiac and vascular function during hemodilution with Dx2M, implying that increased shear stress by HVPE is possibly transmitted to endothelial cells by other remaining components of glycocalyx such as heparan sulfate, chondroitin sulfate and sialic acid or directly transmitted through plasma membrane. Thus, this finding specifically emphasizes that hyaluronic acid plays an important role in the interaction with PEG-HSA, not with HVPE. However, our study did not clearly show the effect of endothelial glycocalyx degradation on shear stress-induced NO synthesis because no significant difference in neither systemic vascular resistance or change in arteriolar diameter was observed between animals treated with and without hyaluronidase. These results are different compared to the observation in ex vivo porcine superficial femoral arteries performed by Kumagai and co-workers.[11] We observed only a small constriction in venules after hyaluronidase infusion followed by hemodilution with PEG-HSA.

Recently, Wang has performed a mathematical model to evaluate the effects of structural change of the glycocalyx on stress distribution and shear rate on endothelial cells.[25] His theoretical solutions have shown that the transmitted stress to endothelial cells by direct pulling of fiber branches of glycocalyx is reduced significantly after degradation of the matrix. Therefore, if PEG-HSA interacts with endothelial glycocalyx, the degradation of hyaluronic acid may attenuate the pulling shear stress on endothelial surface and significantly dominates when hemodilution with PEG-HSA. Furthermore, the pressure gradient in circulatory system was lower, as depicted by lower MAP, after hyaluronidase administration and hemodilution with PEG-HSA, leading to lowering of

total shear stress on endothelial surface. On the other hand, hemodilution with HVPE maintained higher pressure gradient due to higher MAP, implying higher total shear stress on endothelial surface. Thus, hemodilution with HVPE causes higher total shear stress and highly induces endothelial responses and consequential pathways.

In conclusion, this study reveals that PEG-HSA interacts with endothelial glycocalyx via hyaluronic acid and causes beneficial effects on cardiac and vascular function superior from its viscosity. However, the quantification in molecular level of this interaction is necessary to complete the picture how PEG-HSA interacts with hyaluronic acid. In a clinical point of view, the degradation of endothelial glycocalyx in some pathological conditions such as diabetes can be more severe when treated with PEG-HSA or low viscosity plasma expander, leading to endothelial cell dysfunction and depressive cardiac function in the worst case.

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Chapter 7

Conclusions

The work presented in this dissertation was performed to achieve three specific aims: the first dealing with the effects of viscogenic plasma expanders (PEs) on cardiac function, the second related to a contribution of shear stress-induced nitric oxide on cardiac and vascular function and the last dealing with a role of interaction between viscogenic PEs and vascular endothelial surface on cardiac and vascular function. In this chapter, conclusions from the work in each aim are briefly summarized. Furthermore, the strength and limitation of the work are suggested, including future works.

The effects of viscogenic plasma expanders on cardiac function

Our study in an acute hemorrhagic shock and resuscitation demonstrated that increased viscosity with high viscogenic plasma expander (HVPE) such as alginate with viscosity 7.2 cP did not enhance cardiac performance over low viscogenic plasma expanders (LVPEs) such as dextran 70kDa (Dx70; viscosity 2.9 cP) and polyethylene glycol conjugated with human serum albumin (PEG-HSA; viscosity 2.2 cP) in an anesthetized hamsters. This finding is controversial from the studies in microcirculation with the awake hamster window chamber model. The non-beneficial effects of alginate may be, in part, the results of the lower cardiac output, the insufficient energy to eject blood and inhibitory effects of anesthetic, sodium pentobarbital, on sympathetic nervous system in compensatory mechanisms. Despite alginate could not provide a better enhancement compared to other studied plasma expanders, alginate seemly maintained

cardiac function over the observation time after resuscitation while Dx70 gradually decreased in cardiac function as shown by the trend of cardiac function indices such as cardiac output (CO), stoke volume (SV), stroke work (SW) and dP/dt_{max}. Moreover, PEG-HSA demonstrated slightly better effects on cardiac performance compared to Dx70 although its viscosity is lower. Therefore, in this animal model, HVPE has limited beneficial effects on the heart performance after hemorrhagic shock resuscitation compared with LVPEs.

On the other hand, our study in an acute isovolemic hemodilution (Hct 28%) showed that HVPE such as dextran 2000kDa (Dx2M; viscosity 6.3 cP) significantly increased CO, SV and SW compare to baseline. PEG-HSA also efficiently increased CO and preload and lowered pumping work per stroke volume (SW/SV) after hemodilution. We observed that the results in animal hemodiluted with PEG-HSA are in the same direction with that observed in animals hemodiluted with Dx2M and it is better than the results observed in animals hemodiluted with Dx70, although PEG-HSA viscosity is lower. This finding is, in part, in agreement with the findings in an awake hamster window chamber model that PEG-HSA and HVPEs provide beneficial effects in microcirculation during an acute isovolemic hemodilution as enhancing in microvascular perfusion and systemic hemodynamics. Therefore, we suggest that PEG-HSA possibly has the interaction mechanisms with vasculature different from Dx2M but it causes the similar beneficial effects on cardiovascular system.

The contribution of shear stress-induced nitric oxide on cardiac and vascular function

Nitric oxide (NO) is synthesized from endothelial NO synthase (eNOS) activated by shear stress on endothelium. Shear stress can be modulated by changing fluid viscosity or shear rate. Therefore, changing plasma viscosity with different PEs in an acute hemodilution model might influence NO production. Our study shows that Dx2M (HVPE) provided better cardiac function compared to Dx70 (LVPE), including an increase in cardiac output or blood flow and plasma viscosity. These results imply that shear stress is significantly enhanced with Dx2M relative to Dx70, suggesting an increase of endogenous NO synthesized from endothelium and leading to beneficial effects on cardiac function. We tested that increased vascular NO contributes to the enhancement of cardiac function in an acute hemodilution with Dx70. We found that increased vascular NO released by NO donor has beneficial effects on ventricular function by enhancing CO, SV and end-systolic elastance (E_{es}), lowering SW/SV and systemic vascular resistance (SVR) during an acute isovolemic hemodilution with Dx70. We obviously observed these changes in a short period of time, about 15-30 min, after NO donor administration. Despite temporary impact of NO donor, these findings support that shear stress-induced NO, increasing vascular NO availability, has beneficial effects on cardiac function. Furthermore, the experiment with window chamber in awake hamsters showed that there was vasodilation after NO donor infusion, evidently indicating a reduction in vascular resistance which decreased afterload to the heart.

A role of interaction between viscogenic plasma expanders and vascular endothelial surface on cardiac and vascular function

Although PEG-HSA has lower viscosity than Dx2M, they showed similar beneficial effects on microvascular function and cardiac function during an acute isovolemic hemodilution in awake hamsters with window chamber and anesthetized hamsters, respectively. We speculated that the mechanism or interaction between PEG-HSA may couple with endothelial glycocalyx proposed as mechanotransducer, unlike Dx2M. Using hyaluronidase, a hyaluronic acid degradation enzyme, by i.v. infusion followed by hemodilution with PEG-HSA significantly decreased P_{es} and attenuated dP/dt_{max}, dP/dt_{min} and SW compared with hemodilution with Dx2M. These results suggest that hyaluronic acid contributes in the interaction with PEG-HSA but not Dx2M. Furthermore, observation in an awake hamster window chamber model elucidates that endothelial glycocalyx degradation decreased functional capillary density (FCD) at pre-hemodilution state and hemodilution with PEG-HSA after endothelial glycocalyx degradation still decreased FCD. On the other hand, hemodilution with Dx2M maintained FCD nearly same as baseline level after endothelial glycocalyx degradation. Our results did not clearly show vasoconstriction due to impairment of shear stress-induced NO synthesis affected by endothelial glycocalyx degradation. Thus, the beneficial effects on cardiac and vascular function with PEG-HSA as plasma expander in acute hemodilution is not only a result of its viscosity but also the interaction between PEG-HSA and hyaluronic acid, a component of endothelial glycocalyx.

Strengths of this study

Using a miniaturized pressure-volume (PV) conductance catheter in our study provide a feasible approach to assess cardiac function during changing blood and plasma viscosity, providing more understand on how the heart performs to response when blood viscosity changes. Furthermore, this PV conductance catheter has a high fidelity for pressure measurement. The measurement of the heart performance using this technique is independent from heart rate and loading condition. This study provides technical information to design a novel plasma expander based on rheological properties and oxygen carrier-independent idea.

Weaknesses and limitations of this study

In this study, hamsters were anesthetized with pentobarbital by i.p. injection. Generally, anesthetics are cardiac depressive agents. Furthermore, each animal varies to response to anesthetics in different manners. Therefore, it is necessary to judge and monitor animal's conditions such as MAP and HR during the experiment to get the stable conditions independent from anesthetic effects. The other general anesthetics such as inhaled agents may cause less effect on cardiac performance than pentobarbital. Animal under anesthesia is necessary to have intubation to facilitate animal's breathing. Furthermore, animal's core body temperature is also important to monitor and control. Hypothermia affects cardiac chronotropy, leading to lower heart rate.

As the PV conductance catheter generates electric field in the cardiac chamber, not only blood in the cavity but the surrounding tissues of the cavity also contribute in the volume measurement as a parallel volume (V_p). To determine V_p, a bolus of hypertonic saline was intravenously injected at the baseline and end of experiment. However, V_p

varies in each animal and was claimed not high reliability determined by hypertonic saline infusion. Furthermore, volume measurement using the PV conductance catheter is sensitive to the position and orientation of the catheter. The catheter is recommended to align at the center of and straight in the cavity to obtain high accuracy. In an acute hemodilution study, the baseline blood conductance was approximated from measured Hct by the relationship between Hct and blood conductance from pool data. Thus the baseline conductance may not be identical with the value from a direct measurement.

Future directions

The coupling between systemic vascular effects and cardiac function by modulation of plasma viscosity with viscogenic PEs in a condition of decreased Hct , for example hemorrhagic shock resuscitation and isovolemic hemodilution, still need to explore in other view points to put a piece of information for better understanding. We suggest the possible interesting topics as the following:

1) To quantify endothelial NO synthase (eNOS) expression in the heart and vasculatures as well as to measure NO production in blood stream.

2) To study the effects of degradation of other components of endothelial glycocalyx such as heparan sulfate, chondroitin sulfate and sialic acid on cardiac and vascular function.

3) To evaluate the oxygen consumption related to changing of blood or plasma viscosity in a condition of decreased Hct in view of cardiac mechanoenergetic.

4) To study the optimum point between the viscosity of HVPEs and the reduction of Hct that still provide beneficial effects on cardiac function in both cases of hemorrhagic shock resuscitation and acute hemodilution in anesthetized animals.

Appendix I

Mathematical Relationship between Stroke Work and Fluid Viscosity

If we assume the pressure-volume (PV) loop has a rectangular shape as shown in Figure A1.1, stroke work (SW) can be determined from the area of the rectangle. That means

$$SW = (P_{es} - P_{ed}) \cdot (V_{ed} - V_{es}) \tag{A1.1}$$

Assuming Poiseuille flow in the aorta, cardiac output (CO) can be expressed by equation A1.2.

$$CO = \frac{MAP \cdot \pi r^4}{8\mu L} \tag{A1.2}$$

Where MAP is systemic mean arterial pressure, r is vascular diameter, μ is fluid viscosity and L is vascular length. Furthermore, CO can be approximately calculated from stroke volume (SV) and heart rate (HR) as addressed in equation A1.3.

$$CO = SV \cdot HR \tag{A1.3}$$

Stroke volume is the volume difference between diastole and systole of the cardiac cycle, therefore, equation A1.3 can be written into equation A1.4.

$$CO = (V_{ed} - V_{es}) \cdot HR \tag{A1.4}$$

Equation A1.2 is equal to equation A1.3, and then we get the relationship between V_{ed}-V_{es} and fluid viscosity (μ).

$$(V_{ed} - V_{es}) = \frac{MAP \cdot \pi r^4}{8\mu L \cdot HR} \tag{A1.5}$$

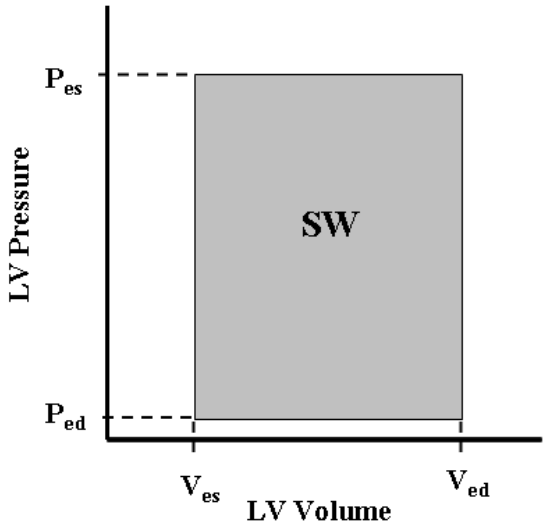

Figure A1.1: Theoretical pressure-volume loop

By substitute equation A1.5 into equation A1.1 and then we can derive the relationship between stroke work and fluid viscosity.

$$SW = (P_{es} - P_{ed}) \cdot \frac{MAP \cdot \pi r^4}{8\mu L \cdot HR} \qquad \text{(A1.6)}$$

By this relationship, stroke work decreases when fluid viscosity is higher. This relationship is in line with the result in Chapter 3 showing that high viscosity plasma expander (alginate) had lower stroke work compare to other plasma expanders. However, this relationship depend not only fluid viscosity but also other parameters such as mean arterial pressure, heart rate and vascular geometry (radius and length).

APPENDIX II

Left Ventricular Blood Volume Estimation

The blood calibration was performed using a series of 4 known-volume cylindrical cuvettes (14.14, 22.09, 31.81 and 43.30 μl). Calibration was established to define Hct effect in blood conductance, as shown in Figure A2.1. Left ventricular blood volume measured by the conductance catheter was determined by using the following equation.

$$V_{lv} = S * RVU + C - V_p \qquad (A2.1)$$

where V_{lv} is the absolute left ventricular blood volume, RVU is the blood conductance measured by PV catheter, S and C are the slope and the intercept of linear regression from blood calibration, respectively, as demonstrated in Figure A2.2 and V_p is the parallel volume caused by the tissues surrounding left ventricle. The baseline RVU values were estimated from baseline Hct of sample blood using a linear relationship between Hct and RVU from pooling data of existing experiment as shown in Figure A2.1.

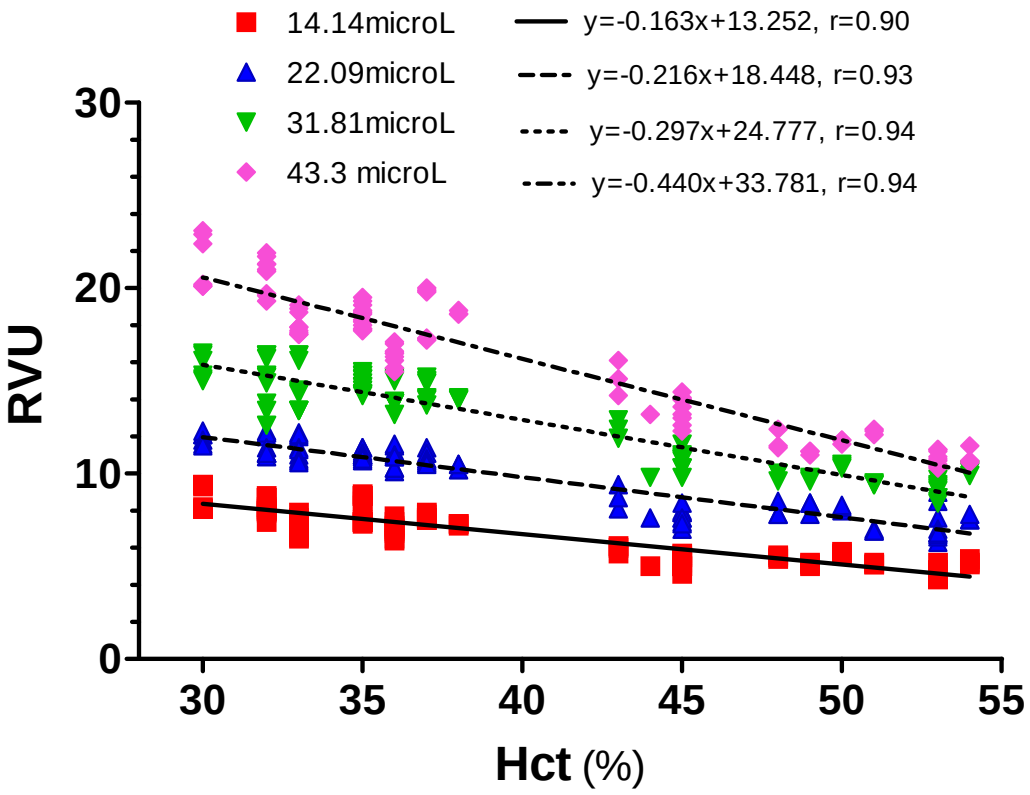

Figure A2.1: Linear regression of systemic hematocrit and blood conductance (RVU) in each known-volume cylinder. This calibration chart was created using our previous experiments (pooling data) in 20 blood samples for each cuvette.

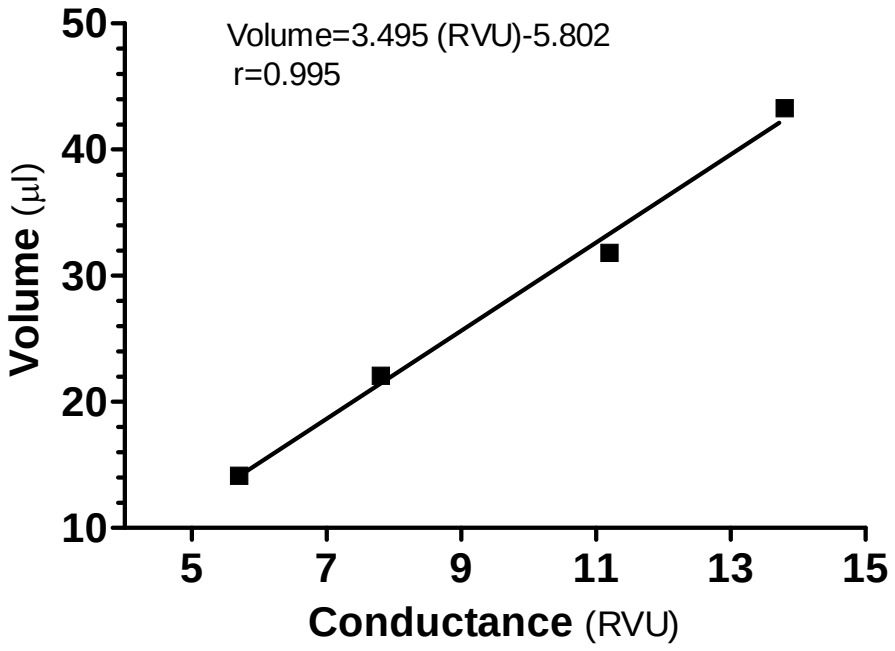

Figure A2.2: Linear regression of blood calibration from blood conductance (RVU) to actual volume (µl) in each known-volume cylindrical cuvette.