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CARDIAC SYSTOLIC FUNCTION RECOVERY AFTER HEMORRHAGE DETERMINES SURVIVABILITY DURING SHOCK

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INTRODUCTION

Hemorrhagic shock results from a large loss of intravascular volume of blood, producing hypotension and hemodynamic abnormalities that lead to the collapse of homeostasis. When hemorrhagic shock becomes established, it leads to anaerobic metabolism, ischemia, heart failure, multi-organ dysfunction and eventually, death.(1–5) Early studies have suggested that cardiac failure is a result of lowering oxygen availability and impairment of myocardial contractile function when hemorrhagic shock is profound.(4, 6–8) Analysis of cardiac function *in vivo* can be complex and challenging during hemorrhagic shock, especially in small animals. 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.(5, 9–13) However, some techniques are not applicable and are costly for small animals. The recent development of a miniaturized conductance catheter provides continuous real time measurement for pressure, volume and their derivatives, high temporal resolution, and more insight to the cardiac function of small animals through pressure-volume analysis for each cardiac cycle.(9, 14–16)

Our objective was to understand and assess left ventricular systolic and diastolic function parameters to establish earlier determinants of survivability after hemorrhage. This study was designed on the premise that the impact of hemorrhage is determined by the overall health condition prior hemorrhage and the amount of blood lost. In this study, all animals were selected from a similar population and within a narrow window of age and weight, and all underwent identical preparation and were exposed to similar stress, if any. To achieve this objective, we performed a study using an anesthetized hamster model. Our experimental model was subjected to a hemorrhage of 40% of blood volume (BV) and followed over 30 minutes during the hypovolemic shock. A miniaturized conductance catheter was used to measure left ventricular pressure and volume during the fixed volume hemorrhagic shock protocol. Derived from the pressure-volume measurements, cardiac performance was evaluated using indices of systolic and diastolic function. In addition, the work done by the heart to pump out blood from the left ventricle was evaluated per ejected blood volume.

METHODS

Animal preparation

Investigation was performed in anesthetized 60 – 70g male Golden Syrian hamsters (Charles River Laboratories, Boston, MA). Animal handling and care followed the NIH Guide for Care and Use of Laboratory Animals and Position of the American Heart Association on Research Animal Use. 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. Tracheotomy was performed and cannulated with a polyethylene-90 (PE-90) tube to facilitate spontaneous breathing. Animals were placed in the supine position and core body temperature was maintained using a heating pad. Animals who responded to a toe pinching during experiment received a small dose of sodium pentobarbital (10–15mg/kg, i.p.). The toe pinching test was performed at less every 5 min to prevent animal discomfort. The use of anesthetics is often inevitable because of the invasiveness of the experimental procedure needed.

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: i) mean arterial blood pressure (MAP) above 80 mmHg, ii) heart rate above 320 beats/minute and iii) systemic hematocrit (Hct) above 45%.

Systemic parameters

The MAP and HR were monitored continuously (MP150, Biopac System Inc., Santa Barbara, CA). Hct was determined from centrifuged arterial blood samples taken in heparinized capillary tubes (50µl). Hemoglobin (Hb) content was measured by spectrophotometer (B-Hemoglobin, Hemocue, Stockholm, Sweden).

Cardiac function

Closed chest method was performed to assess cardiac function in this study.(14) 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 (LV). The PV catheter provided the instantaneous pressure and volume information of the left ventricle. The PV catheter provided the instantaneous pressure and volume information of the left ventricle. Parallel volume (V_p) at baseline and the end of the experimental protocol were determined by bolus intravenous injection of 15% hypertonic saline (10 µl, 0.25 % of the BV). A hypertonic saline injection was used to create a transient drift in the conductivity of blood to establish the parallel current through adjacent structures. The V_p was determined by an intersecting point of the linear regression line of end-systolic (V_{es}) on end-diastolic (V_{ed}) volume with the line of identity ($V_{es}=V_{ed}$) as performed in previous studies.(14–16)

Hemorrhagic shock protocol

Animals were anesthetized and after a 20 min stabilization period, 40% of estimated blood volume (BV) was withdrawn via the femoral artery catheter within 15 min. The total BV was estimated as 7% of body weight. The shock condition was held for 30 min to monitor systemic condition and to investigate cardiac function. Systemic parameters (MAP, HR, Hb and Hct) were recorded and analyzed at baseline and 30 min after shock as schematically shown in Figure 1. The animals were considered *non-survival* if suddenly died or their MAP decreased below 30 mmHg over a 5 min period.

Estimation of left ventricular blood volume

Left ventricle 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. RVU to blood volume 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. Blood volume measured by the conductance catheter was determined by using the following equation 1.

$$V_{lv} = S \times RVU + C - V_p \quad (1)$$

where V_{lv} is the absolute left ventricular volume; RVU is the blood conductance measured by PV catheter; V_p is the parallel volume; S and C are the slope and the intercept of linear regression from blood calibration.

Estimation of hematocrit during and after hemorrhage

The electrical conductivity of blood depends on the concentration of red blood cells (17). Therefore, in order to determine the blood conductance for calibration without taking additional blood samples from the hemorrhaged animals, systemic Hct during and after blood loss was estimated based on the equation proposed by Bourke and Smith (18), equation 2.

$$Hct_f = Hct_i \times e^{-(EBL/EBV)} \quad (2)$$

where Hct_f is Hct at the time point of interest; Hct_i is the initial Hct (baseline Hct); EBV is the estimated blood volume at the baseline; and EBL is the estimated blood loss.

Data analysis of cardiac function

Cardiac function data were analyzed with PVAN software (version 3.6, Millar Instruments, TX). At the baseline, pressure-volume loops were selected if they had four basic phases: ventricular filling, isovolumic contraction, ejection and isovolumic relaxation. Indices of systolic and diastolic function were calculated including maximum rate of pressure change (dP/dt_{max}), ratio between dP/dt_{max} and end-diastolic volume ($dP/dt_{max}/V_{ed}$), left ventricular end-systolic pressure (P_{es}), minimum rate of pressure change (dP/dt_{min}), left ventricular end-diastolic pressure (P_{ed}) and left ventricular relaxation time constant (Tau). Stroke work (SW) was also normalized by stroke volume (SV) representing the work performed 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.

Statistical analysis

Results are presented as mean and SD unless otherwise noted. The values are presented as relative to baseline, a ratio of 1.0 signifies no change from baseline, whereas lower or higher ratios indicate reductions or increases from baseline. Results are presented as means $\pm$ SD unless otherwise denoted. The Grubbs' method was used to assess closeness for all measured parameters values at baseline. This method quantifies how far each parameter value is from the other values obtained, computing a P value supposing that all the values were really sampled from a Gaussian population. Data in both groups were analyzed using two-way ANOVA nonparametric repeated measurements, and, when appropriate, post hoc analyses to baseline were performed with the Bonferroni post tests. The product limit method (Kaplan-Meier) was used to produce survival curves, and analysis of survival was conducted using the log-rank test. All statistics were calculated using GraphPad Prism 4.01 (GraphPad Software, San Diego, CA). Results were considered statistically significant if $P < 0.05$.

RESULTS

Survival

Thirteen animals were included in the study, all animals survived the hemorrhage. All animals included in the study passed the Grubbs' test ensuring that all the measured parameter values at baseline were within a similar population ($P < 0.05$). Survival rate during the 30 min period of hypovolemic shock was 61.5% (8 of 13 animals). Survival group (**S**; $n=8$, 64.3 ± 3.3 g) and Non-survival group (**NS**; $n=5$, 65.4 ± 2.9 g) were not different in body weight. Four animals in the NS group died 30 min after hemorrhage (SH30). The difference in survival between these 2 groups was statistically significant ($P < 0.05$). Retrospective analysis of sodium pentobarbital anesthesia administer post animals post surgical preparation indicates that **S** received two doses of 10–15 mg/kg of pentobarbital (first 10–15 min, and second 25–30 min after hemorrhage, respectively), and **NS**, who only received one (10–15 min after hemorrhage). Moreover, analysis MAP and HR showed only less than a 10% changed on these parameters.

Systemic parameters

Figure 2A presents changes in MAP for S and NS groups. Hemorrhage drastically decreased MAP in all animals to 35 mmHg after hemorrhage. The S group recovered MAP during hypovolemic shock. The NS group MAP slightly increased within the first 15 min of shock. MAP during shock between S and NS groups was significantly different at 25 min and 30 min ($P < 0.05$). Figure 2B shows the changes in HR for S and NS groups. HR decreased after hemorrhage in both groups. During hypovolemic shock, HR was maintained over time in the survival group, while in the NS group it continually decreased.

Cardiac function

The maximum rate of pressure change ($dP/dt_{\max}$), gradually increased after hemorrhage in S group. Furthermore, $dP/dt_{\max}$ was significantly different between S and NS groups at 15, 20, 25 and 30 min of shock as shown in Figure 3A ($P<0.05$). At 30 min shock, $dP/dt_{\max}$ of non-survival animals was about 50% of the survival animals' value.

When considered a load-independent contractility index, the ratio of $dP/dt_{\max}$ and V_{ed} , in the shock phase was found to be higher than the baseline value in the S group, Figure 3B. On the other hand, they were lower than the baseline in the NS group. NS animals presented impaired contractile function, as the load-independent contractility index gradually decreased over time. At 30 min of shock, $dP/dt_{\max} / V_{ed}$ in the non-survival group was about 44% of the survival group level.

Left ventricular end-systolic pressure (P_{es}) was found in both groups reduced post hemorrhage, Figure 3C. P_{es} for the S group, significantly improved during the shock. P_{es} between the S and NS groups were statistically significant 30 min after hemorrhage ($P<0.05$).

In the S group, the minimum rate of pressure change ($dP/dt_{\min}$) significantly recovered during the shock Figure 4A. In the S group, $dP/dt_{\min}$ improved during shock compared to the NS group. Most of the animals in NS group failed when $dP/dt_{\min}$ was decreased to 51% of the value in the S group. However, when we considered the end-diastolic pressure (P_{ed}) and the relaxation time constant (τ) which were diastole-related indices, we found that there was no significant difference between S and NS groups, Figure 4B and 4C.

The work performed by the heart per stroke volume (SW/SV) can be used to assess the cardiac performance. This ratio gave the information about how much work was produced by the heart per total ejected volume. As shown in Figure 5, it was found that the ratio SW/SV significantly improved between the end of hemorrhage and the end of shock in the S group ($P<0.05$). Conversely, this improvement was not observed in the NS group. The SW/SV in the S group was also significantly different from the NS group since 20 min within the shock period ($P<0.05$), and animals failed in the NS group when SW/SV was about 75% of the value in the S group.

DISCUSSIONS

This study analyzed left ventricular cardiac function in survival and non-survival animals post hemorrhage during the hypovolemic shock. We found that systolic and diastolic function gradually improved after hemorrhage in survival animals while non-survival animals failed to recover cardiac function in both systole and diastole phases (Figure 6A and 6B). According to our findings, the impairment in systolic function showed more significant influence on mortality, than the deficit in diastolic function. The reduction in systolic function influenced the ejection phase and led to a systemic cardiovascular collapse as presented by the gradual decrease in MAP (60% decrease in MAP from baseline) in non-survival animals. Ventricular pressure measurements have been commonly used for decades, but real-time volume measurements have historically been problematic. For a small rodent,

the conductance catheter offers unique advantages over all other available approaches to measure cardiac function. It enables more specific measurement of the LV performance independent of loading conditions, heart rate and diastolic function.

Systolic function evaluated by load-dependent indices ($dP/dt_{\max}$ and P_{es}) progressively decreased during hemorrhage in both groups. Previous studies using biplane cinefluorography to assess left ventricular contractility presented similar results for hypovolemic shock in dogs.(10) The diastolic function drastically decreased from the baseline during hemorrhage, and during hypovolemic shock, as shown by the decrement of $dP/dt_{\min}$ and P_{ed} and the increment of τ . Assessment of contractility with load-dependent indices may limit their values and partially reflect the systolic performance.(19) However, $dP/dt_{\max}$ have been widely used as indices of contractility.(10, 20–22) In this study, we minimized the preload effect on $dP/dt_{\max}$ by normalizing it by V_{ed} . Interestingly, $dP/dt_{\max}/V_{ed}$ in the survival group was higher compared to baseline, while it was lower than baseline for the non-survival group. These results imply that contractility was increased, due to the compensatory responses to hypotension/hypovolemia in the survival group. In addition, work performed per stroke volume by the heart, decreased during hemorrhage in the non-survival animals, and worsened cardiac performance and energy transduction, from the heart to the blood.

As with our results in the survival group, MAP continually increased over the shock period, and significantly improved from the end of hemorrhage, indicating the autonomic response to the hypotension was activated in order to recover the systemic conditions. Studies in an awake hamster window chamber model showed that vasoconstriction occurred in the shock phase and correlated with increasing MAP; this may explain the increased MAP during shock in the survival group.(23, 24) On the other hand, failing to restore MAP after hemorrhage possibly indicated the high risk in mortality as shown in non-survival animals. The failure in MAP restoration causes failure of peripheral circulation and tissue oxygenation.

During the shock period, left ventricular contractility evaluated by $dP/dt_{\max}$ improved rapidly after hemorrhage, despite no fluid resuscitation in survival animals, and recovered toward to baseline. The $dP/dt_{\max}/V_{ed}$ was also apparently enhanced after hemorrhage in the survival group. The previous study by Welte *et al.* showed that, during the shock period, the contractility assessed by the slope of the end-systolic pressure volume relationship (ESPVR) significantly increased in response to hemorrhage.(12) Non-survival animals developed $dP/dt_{\max}$ and $dP/dt_{\max}/V_{ed}$ in the early stages of shock (SH0 to SH10). However, these indices decreased over the remaining shock period in the non-survival group, indicating a progressive impairment in the pressure development to pump out blood from the heart, and the reduction of cardiac contractility.

The rapid recovery of end-systolic pressure (P_{es}) to baseline after hemorrhage clearly indicated the vital sign of survival, and correlated with the increased MAP. The increased P_{es} implies that the afterload also increases as a result of vasoconstriction. The improved P_{es} , after hemorrhage, may lead to an increase in coronary pressure, and consequently

improve coronary blood perfusion. Thus, cardiac function is improved and restored to baseline.

Cardiac function in diastole assessed by $dP/dt_{\min}$ demonstrated a change in similar direction as a change in $dP/dt_{\max}$ for both survival and non-survival animals. The difference in $dP/dt_{\min}$ between groups was less than that found in $dP/dt_{\max}$, because the relaxation and filling phases are passive actions compared to the contraction and ejection phases of cardiac cycle. The time constant during an isovolemic relaxation (Tau) proposed by Weiss *et al.* was also used to assess the diastolic function of the heart during shock phase.(25) It revealed that survival animals had a shorter relaxation phase than non-survival animals had. However, the difference in Tau between these two groups was insignificant. In addition, there is no significant difference in P_{ed} between the survival and non-survival groups. These results support the notion that the deficiency in diastolic function does not play an important role in mortality during hypovolemic shock, unlike the deficiency in systolic function.

Work perform per stroke volume by the heart is a very important indicative parameter to distinguish between the survival and non-survival groups, and to assess cardiac performance during hemorrhagic shock. Survival animals had a significantly higher work accomplished per stroke volume compared with non-survival animals. This finding is similar to the study by Horton *et al.*, that reported significant increases in stroke work, after hemorrhage.(10) Other studies in anesthetized rats have shown an increase in cardiac work during shock, however they did not associate the absence of this mechanism with the hindering of survival, during hypovolemia.(26) HR and MAP are not strong indicators of SW, as both relationships between HR and SW, and MAP and SW do not present a strong correlation in survival or non-survival groups. The double product (HR*MAP) compared to baseline, linearly correlated to SW. This double product indirectly represents myocardial oxygen consumption. Considering the results about the work performed by the heart, moderate hemorrhagic shock disrupts myocardial energy conversion from oxygen molecules to the mechanical work. However, the study in hemorrhagic hypotensive rats by Kline *et al.* reported that the hemorrhagic hypotension impaired cardiac function but did not markedly change ATP concentrations in ventricular myocardium.(26) This delineates a basis for future studies to guide therapeutic interventions in patients experiencing hemorrhagic shock, other than primarily restoring preload.

The degree of hemorrhagic shock depends on the volume of blood loss, and the duration of the hypotension or shock period.(26, 27) To interpret any hemorrhagic shock experimental results, it is necessary to understand that each animal specie and anesthetic used, affects the outcome of the study.(11, 14) Our study was carried out using a systematic hemorrhage of 40% of BV in anesthetized animals, and the hypovolemic shock was maintained for 30 min. Studies using the technique used by us, here, had found considerable differences in cardiac function with different anesthetic agents.(14) Various animal models of heart failure may be more sensitive to the cardio-depressive effects of injectable agents, and each animal specie seems to respond differently.(28, 29) Previous investigations in hamsters have shown that pentobarbital anesthesia produces stable cardiac, lung, and airways mechanics, which allows for long-term studies.(30, 31) In our study, the dose of sodium pentobarbital during surgical preparation was five times higher than during the experiment if the animals responded to a

toe pinching. Retrospective analysis of the out-come and anesthesia, indicates that survival correlates with higher doses of anesthesia received. Therefore, we can conclude that pentobarbital hemodynamic depression did not influence the results. The cardio-depressive effects of pentobarbital, if any, were similar in both groups, and their differences in cardiac function were mostly caused by the dysfunction produced by the hemorrhagic shock.

Natural tolerance to identical bleeding volume can result from genetic variations, and the results shown here, relate to down stream effects produced by genotypic and/or autoregulatory mechanisms differences between survival and non-survival animals. The inter-individual differences after hemorrhage may be genetic in origin, as hemorrhage-induced ischemia is a potent stimulus for gene expression. Consequently, functional parameters are affected by the synthesis of gene products. Within the limited number of variables studied, the differences between survival and non-survival correspond to: i) recovery of systolic function, or ii) absence of severe physiologic insult on systolic function. In conclusion, the impairment of systolic functional recovery, after hemorrhage, is the major determinant of mortality in non-survival animals, not the deficit in diastolic functional recovery. The failure to restore blood pressure clearly indicates a high risk of non-survival. Thus, positive development in left ventricular pressure by enhancing contractility or preload, during hemorrhagic shock can effectively increase the survivability.

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Figure 1.

Schematic diagram of fixed volume-hemorrhagic shock protocol. BL, baseline; SH30, 30 minutes after beginning of shock. BV, blood volume.

Figure 2.

(A) Mean arterial pressure (MAP) measured at baseline (BL), at beginning of shock (SH0), at 5, 10, 15, 25 and 30 minutes after beginning of shock (SH5, SH10, SH15, SH20, SH25, SH30). Values are presented as means $\pm$ SD. †, $P < 0.05$ between groups. (B) Heart rate measured at baseline (BL), at 5, 10 minutes after beginning of hemorrhage (H5, H10), at beginning of shock (SH0), at 5, 10, 15, 25 and 30 minutes after beginning of shock (SH5, SH10, SH15, SH20, SH25, SH30). Values are presented as means $\pm$ SD. †, $P < 0.05$ between groups.

Figure 3.

Left ventricular systolic function indices derived by PV conductance catheter. **(A)** Maximum rate of pressure change (dP/dt_{max}) during and 30 minutes after hemorrhage. **(B)** Ratio of maximum rate of pressure change and end-diastolic volume ($dP/dt_{max}/V_{ed}$) during and 30 minutes after hemorrhage. **(C)** Left ventricular end-systolic pressure (P_{es}) at baseline, during and 30 minutes after hemorrhage. Broken line represents baseline level. Values are presented as means $\pm$ SD. †, $P < 0.05$ between groups. Time points as in Figure 2.

Figure 4.

Left ventricular diastolic function indices derived by PV conductance catheter. **(A)** Minimum rate of pressure change (dP/dt_{min}) during and 30 minutes after hemorrhage. **(B)** Left ventricular end-diastolic pressure (P_{ed}) at baseline, during and 30 minutes after hemorrhage. **(C)** Left ventricular relaxation time constant (Tau), during and 30 minutes after hemorrhage. Broken line represents baseline level. Values are presented as means $\pm$ SD. $\dagger$, $P < 0.05$ between groups. Time points as in Figure 2.

Figure 5.

Left ventricular work perform per stroke volume. Broken line represents baseline level.

Values are presented as means $\pm$ SD. †, $P < 0.05$ between groups. Time points as in Figure 2.

Figure 6. Pressure-Volume (PV) loops at the baseline (BL), at the beginning of hypovolemic shock (SH0) and at 15 and 30 min during shock (SH15, SH30) in (A) survival animal and (B) non-survival animal.