SHOCK, Vol. 28, No. 4, pp. 426–433, 2007

UNCONTROLLED HEMORRHAGE DIFFERS FROM VOLUME- OR PRESSURE-MATCHED CONTROLLED HEMORRHAGE IN SWINE

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Received 12 Oct 2006; first review completed 3 Nov 2006; accepted in final form 11 Jan 2007

ABSTRACT—Controversy continues as to whether uncontrolled or controlled hemorrhage is the most appropriate for the study of hemorrhagic shock and resuscitation. To appraise differences between these models, we evaluated the relationship between blood volume loss and blood pressure in controlled versus uncontrolled hemorrhage. Anesthetized, instrumented, immature female pigs (40 kg) were assigned to one of three groups: (1) group U, uncontrolled aortotomy hemorrhage from a 2-mm aortotomy; (2) group P, controlled hemorrhage matched to the blood pressure profile of group U; or (3) group V, controlled hemorrhage matched to the blood volume loss profile of group U. A computer-driven feedback control system duplicated the group U profiles. Pigs were monitored for 3 h after hemorrhage and received no fluid resuscitation. Group U resulted in a blood loss of 17.6 ± 0.7 mL kg^{-1} and a reduction in blood pressure to 28 ± 3 mmHg at the end of active bleeding. Group P pigs required more blood loss (21.5 ± 1.2 mL kg^{-1}) to match profiles of group U blood pressure, whereas group V pigs resulted in a higher mean arterial pressure (42 ± 5 mmHg) to match group U blood volume loss profiles. Neither heart rate nor total peripheral resistance differed significantly among the three groups. At the level of blood loss observed in this study, fundamental physiological differences existed between uncontrolled hemorrhage and controlled hemorrhage when matched for pressure or volume. We suggest that the relationship of blood pressure to blood volume loss is modified in the presence of uncontrolled hemorrhage.

KEYWORDS—Aortotomy, controlled hemorrhage, hemodynamics, lactate, pigs

INTRODUCTION

A large body of literature has accumulated about the physiological responses and evaluations of various interventions in hemorrhage. These studies have used different models in a variety of animal species, but controversy about the reproducibility and control of such hemorrhage models versus their clinical relevance continues.

Current models of hemorrhage fall into two general categories: controlled or uncontrolled. Generally, controlled hemorrhage models have been of two types: either the animal is bled to a fixed blood pressure or a fixed volume of blood is removed (1). The greatest advantage of controlled hemorrhage is its reproducibility across a number of species. However, although these controlled hemorrhage models have been useful for comparing different treatment strategies, they may fail to truly simulate many aspects of hemorrhagic hypotension seen in trauma victims (1).

Alternatively, a number of investigators have developed animal models of uncontrolled hemorrhage by inducing an arterial (2) or solid organ injury (3, 4), or by simply cutting an appendage (5, 6). Proponents of models of uncontrolled hemorrhage have claimed that these models are more clinically relevant because only the animal's normal hemostatic mechanisms can intervene with the progression of the hemorrhage, and also because the models incorporate some degree of tissue trauma. However, in most models of uncontrolled hemorrhage, there is increased variability in the extent of bleeding, inducing inconsistency in outcome measures such as reduction in blood pressure and survival.

Of interest in uncontrolled hemorrhage models is the seemingly disproportionate reduction of blood pressure for the magnitude of blood loss compared with controlled hemorrhage, possibly due to the presence of tissue injury. In their early study, Bickell et al. (2) made this observation in the aortotomy model of uncontrolled hemorrhage in conscious animals. In their study, a pronounced reduction in blood pressure was noted within seconds of injury and well before there was a significant reduction in blood volume. Similar differences have been noted in pilot studies performed in our laboratory. For example, in a fixed pressure model of hemorrhage in swine, a MAP of 30 to 35 mmHg was attained with a blood loss of approximately 30 mL kg^{-1} (7). In contrast, using an uncontrolled hemorrhage model, a similar reduction in blood pressure was attained with a blood loss of only 20 mL kg^{-1} (8). Similar levels of blood loss (20 mL kg^{-1}) and blood pressure (30 mmHg) to those in aortotomy-induced uncontrolled hemorrhage were also observed after a grade V liver injury in pigs (9). In response to ballistic injury in goats, there was a rapid reduction in blood pressure from 105 to 70 mmHg with a blood volume loss of only 3.8 mL kg^{-1} (10), a volume previously shown not to elicit a change in blood pressure in controlled hemorrhage (personal observations). Thus, although there is a tight and predictable interrelationship between blood volume and blood pressure in controlled hemorrhage, it appears factors other than loss of blood volume may influence blood pressure regulation in response to uncontrolled hemorrhage. These other factors must

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This study was supported by the US Army Medical Research and Materiel Command.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense (AR 360-5).

DOI: 10.1097/shk.0b013e31804a5791

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 OCT 2007	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Uncontrolled hemorrhage differs from volume- or pressure-matched controlled hemorrhage in swine	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
Sondeen J. L., Dubick M. A., Holcomb J. B., Wade C. E.,	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX 78234	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**8**	

be understood if translation from preclinical models to the clinic can reliably occur.

The objective of the present study was to determine whether the difference observed retrospectively between studies of controlled and uncontrolled hemorrhage would be similarly observed in a prospective evaluation of these hemorrhage models. We evaluated the hemodynamic and metabolic changes in swine subjected to uncontrolled hemorrhage (vascular injury), and compared and contrasted these changes to those in swine subjected to controlled hemorrhages that mimicked either the blood pressure profile or the blood volume loss of uncontrolled hemorrhage over time. We hypothesized that (1) animals subjected to a fixed-pressure controlled hemorrhage, bled to match the blood pressure profile of animals subjected to uncontrolled hemorrhage, would experience greater blood loss as compared with animals in the uncontrolled hemorrhage group; and (2) animals subjected to a fixed-volume controlled hemorrhage, bled to match the volume loss profile of animals subjected to uncontrolled hemorrhage, would experience a smaller drop in blood pressure as compared with animals in the uncontrolled hemorrhage group.

MATERIALS AND METHODS

Experimental procedure

All animals were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care International–accredited facility. The protocol was approved by the Animal Care and Use Committee of the US Army Institute of Surgical Research. All animals received care in compliance with the *1996 Guide for the Care and Use of Laboratory Animals* by the National Research Council. Immature female Yorkshire pigs (39.8 ± 0.4 kg) were obtained from a local vendor (HDH Swine Farm, Boerne, Tex). The day before an experiment, the pig was isolated in a single cage and fasted overnight with water available *ad libitum*. Animals were premedicated by an injection of glycopyrrolate (0.005–0.01 mg kg^{-1}, i.m.) for controlling secretions and Telazol (4–6 mg kg^{-1}) for sedation. After tracheal intubation, they were placed on mechanical ventilation with 100% oxygen and anesthetized with 2% to 3% isoflurane and ketamine (100 μg kg^{-1} min^{-1}, i.v.). A lateral neck incision was made, and polyvinyl chloride catheters were placed in the thoracic aorta via the carotid artery for blood pressure and heart rate (HR) monitoring. One radiopaque sheath introducer (8.5F; American Edwards Laboratory, Inc., Irvine, Calif) was inserted into the external jugular vein, and a Swan-Ganz thermodilution catheter (7F; American Edwards) was inserted through the sheath to the wedge position in the pulmonary artery for recording pressures. A second neck incision was made, and Teflon Angiocath i.v. catheters were inserted nonocclusively into the carotid artery and jugular vein. A Paratrend catheter (Diametrics Medical, High Wycombe, Buckinghamshire, UK) was then inserted through each Angiocath for continuous blood gas measurements. A left thoracotomy at the fourth intercostal space was performed, and an ultrasonic flow probe (Transonic Systems, Ithaca, NY) was placed around the ascending aorta to determine cardiac output (CO). The chest wall was then closed by standard suture procedures. Care was taken to inflate the lungs fully as the chest wall was closed to exclude all air; however, no chest tube with negative pressure was used. After a midline ventral celiotomy, the spleen was removed after double ligation of all vascular pedicles to eliminate the effects of autotransfusion induced by a contractile spleen on the hemodynamic and metabolic variables measured. The spleen was immediately weighed and the animal infused with warm lactated Ringer solution at three times the splenic weight to replace the volume of blood contained in the removed spleen. A large-bore catheter (radiopaque sheath introducer, 8.5F; American Edwards) for arterial sampling and hemorrhage was inserted occlusively into the iliac artery and its tip advanced into the abdominal aorta past the bifurcation. The catheter tip was placed near the site of the aortotomy so that the controlled hemorrhage would be from a site similar to the uncontrolled hemorrhage, but the tip was distal from aortotomy site so that it did not disturb the clot in the animals receiving an aortotomy when arterial samples were drawn. The abdominal incision was kept open for access to the aorta and for the suction tubes, but a plastic-coated sheet was placed over the site to minimize heat and evaporative water loss. Animals from all groups were instrumented identically. Electrocardiogram leads were placed on the pig's limbs and chest. Arterial, central venous, right ventricular, and pulmonary artery pressures were monitored continuously using strain gauge pressure transducers (model P23XL; Gould Instruments, Oxnard, Calif) and the measurements recorded using a Gould polygraph (Gould Instruments, Valley View, Calif). All analog data were continuously acquired on the MI2 system (Modular Instruments, Inc., Malvern, Pa). All digital data were acquired using the Software Wedge RS232-serial communication software packages (TAL Technologies, Inc., Philadelphia, Pa). After the instrumentation was completed and the animal stabilized, a 10-min baseline period was begun for recording hemodynamic and metabolic measurements. Animals were then matched to one of three groups described below. In addition, three pigs were included as time controls to assure stability of the anesthetic regimen used (data not reported). Animals were monitored for 3 h after the end of hemorrhage or until death. Those animals surviving to 3 h were euthanized with a euthanasia solution (Fatal Plus, Fort Dodge, Iowa).

Uncontrolled hemorrhage (U)

Pigs (n = 6) in the uncontrolled hemorrhage group U had perforated sleeves (ViaGuard sump suction converter sleeve; SurgiMark, Inc., Yakima, Wash) laid along the lateral dorsal abdominal walls bilaterally and suction tubes placed in the sleeves. In this way, the intestines did not cover the tips of the suction tubes, and blood was suctioned using a Vac-Rite disposable suction system (Baxter, Deerfield, Ill) placed onto a balance (SR16000 Mettler Balance; Mettler-Toledo, Highstown, NJ) to capture blood lost from the aortotomy. A third handheld suction tube was used to capture any blood that appeared among the intestinal loops. With gentle retraction of the intestines, the infrarenal aorta was exposed, and a hole was made with a 2.0-mm disposable skin biopsy punch (Acuderm, Inc., Ft. Lauderdale, Fla). The intestines were then restored to their natural position over the injury site. The skin punch plugging the hole was removed to commence the bleeding. The aortotomy bleeding was allowed to spontaneously stop, which usually occurred in 4 to 6 min. Blood volume lost and pressure were continuously recorded throughout the hemorrhage and experimental period. After the animal was dead, the abdominal cavity was explored for any pooled clotted blood that did not suction into the containers on the balance originally because retained blood would invalidate the pressure volume relationship. Individual animal profiles were used to model blood loss or blood pressure reductions for the subsequent experiments of controlled hemorrhage.

Controlled hemorrhage

Experimental duplication of the blood volume loss or pressure change of uncontrolled hemorrhage was accomplished with a Masterflex Easy Load II peristaltic rotary infusion pump with computerized drive (Cole-Parmer Instrument Co., Vernon Hills, Ill) using customized LabVIEW software (National Instruments Corp, Austin, Tex). Subsequent animals were assigned to one of two groups: (1) group P (n = 11), with controlled hemorrhage matched to the blood pressure profile curve over time of the uncontrolled hemorrhage animal; and (2) group V (n = 11), with controlled hemorrhage matched to the blood volume loss profile over time of the uncontrolled hemorrhage animal. At 10-s intervals, the mass (in grams) of total blood loss was weighed on a balance with simultaneous measurements of MAP. The blood loss weight was converted to volume (in milliliters) using an average specific gravity of 1.0384 g ml^{-1}, determined by weighing 10 aliquots of precisely measured (10 mL) pig blood using a single plastic syringe at room temperature. The data were recorded into the computer data file so that profiles of accumulated blood volume (in milliliters), flow rates (in milliliters per minute), and blood pressures could be calculated. The length of time for the simulated uncontrolled hemorrhage was determined by the volume or pressure profile of uncontrolled hemorrhage recordings.

The uncontrolled hemorrhage profile assigned to each controlled hemorrhage animal was based on the initial blood pressure of the animal. From previous experience, we knew that initial blood pressure was correlated with the volume of blood loss during uncontrolled hemorrhage ($r = 0.32$, n = 103, $P < 0.003$; J. L. Sondeen, unpublished observations). To assure this matching of baseline MAP for pigs in groups P and V, some of the uncontrolled hemorrhage profiles were used more than once because their baseline MAP matched the controlled animal's baseline MAP. Some of the uncontrolled hemorrhage profiles were not used if the result had been immediate exsanguination of the animal or if little blood loss occurred, resulting in a nonhypotensive hemorrhage. As a result, six uncontrolled profiles were used to generate 11 paired sets of data.

The experiment was originally designed so that each U would be followed by a randomly assigned P or V. However, it was not possible to control the initial MAP of the V or P pigs within 5 mmHg of each new U pig. A total of 14 uncontrolled hemorrhages were performed randomly throughout the experimental period. Of those animals, only six had MAPs that were able to be matched to one to three animals in the P and V groups, making a total of 11 matched sets.

Arterial and venous blood samples were collected at baseline, at the end of active bleeding, and at 15, 30, 60, 90, 120, and 180 min after the onset of hemorrhage. Baseline blood gases, including pH and base excess, were obtained using an AVL blood gas machine (AVL Scientific, Roswell, Ga). After the initial blood sample for calibration, arterial and venous blood gases were measured continuously using a Trend Care Blood Gas Monitoring System (Diametrics

TABLE 1. **Hemorrhage volume, rate, time, nadir MAP, and survival data**

	U	V	P	*P*
Matched groups (V and P matched to U)				
n = 11				
Hemorrhage volume (ml kg^{-1})	17.6 ± 0.5	17.4 ± 0.5	21.3 ± 1.4*	≤0.0358
Hemorrhage rate (mL kg^{-1} min^{-1})	2.8 ± 0.1	2.9 ± 0.2	5.5 ± 0.6 *	<0.0001
Hemorrhage time (min)	6.3 ± 0.1	6.1 ± 0.2	4.2 ± 0.6 *	≤0.0006
Nadir MAP (mmHg)	29 ± 2	40 ± 5†	32 ± 2	≤0.024
Survival time (min)	151 ± 15	108 ± 11	116 ± 20	NS
Survival rate (%)	36	82	54	NS
Unmatched groups (including nonmatched animals)				
n	14	17	22	—
Hemorrhage volume (mL kg^{-1})	15.9 ± 1.4	16.0 ± 0.8	20.5 ± 0.9*	<0.0001
Nadir MAP (mmHg)	30 ± 2	45 ± 4†	27 ± 1	≤0.002
Survival time (min)	120 ± 19	161 ± 13	116 ± 15	NS
Survival rate (%)	42	88	50	NS

Data expressed as mean ± SE.
*Different from U and V.
†Different from U and P.
NS indicates not significant.

Medical). Complete blood counts, hematocrit, and hemoglobin concentration were determined on a System 9000 Hematology Series Cell Counter (Baker Instruments, Allentown, Pa). Total protein, glucose, creatinine, and lactate were determined by standard clinical chemistries on a Vitros 250 Clinical Chemistry Analyzer (Ortho Clinical Diagnostics, Rochester, NY).

Statistical analysis

Data analyses were carried out using SAS version 8.1 (SAS Institute, Inc., Cary, NC) and SPSS version 10.1.3. The data were compiled into 11 matched sets for comparing P and V to U. The primary end points were MAP for comparison of groups U and V, and blood volume loss at the end of the hemorrhage period for comparison of groups U and P. Data were analyzed by ANOVA, followed by Tukey method for posthoc comparisons. $P \leq 0.05$ was considered statistically significant. Categorical data (i.e., survival) were analyzed by nonparametric tests (Fischer's exact test). Linear regression was used to find the relationship between hemorrhage volume (hemvol) and MAP and between the change in MAP and the change in HR. Linear regression analysis assumes randomness or independence of the data. When regression is performed on time series data, the errors are often not independent. The errors may be autocorrelated, that is, each error is correlated with the error immediately before it. Therefore, the Durbin-Watson *d* statistic was used to test that the autocorrelation was zero (11, 12). Because autocorrelation (*r*) was found to be present in each of the groups, the *x* and *y* variables, for example, hemvol and MAP, were transformed to hemvolT and MAPT by the Cochrane-Orcutt procedure to eliminate the autocorrelation (i.e., dMAPT = dMAP [i] − *r** dMAP [i − 1], hemvolT = hemvol [i] − r × hemvol [i − 1] (11, 12)). In the figures, the dMAPT was then regressed against hemvolT to obtain slope b* and intercept a*, which were translated back to b and a by b = b* and a = a*/(1 − r). ANCOVA was used to test if there were significant differences among the three slopes or intercepts.

RESULTS

Time control

Hemodynamic and metabolic variables remained relatively constant over the 3-h experimental period in the time-control animals, indicating the stability of the model (data not shown).

Uncontrolled hemorrhage

The aortotomy site actively bled for an average of 6.3 ± 0.1 min and resulted in a blood loss of 17.6 ± 0.5 mL kg^{-1} (Table 1). During this period, the animals had a significant reduction in MAP to 28 ± 3 mmHg compared with baseline (44% of baseline; Fig. 1). Heart rate rose and remained significantly elevated over baseline (137%) after the active bleeding period and for the remainder of the experiment (167% at 180 min; Fig. 1). Cardiac output fell to 36% of baseline at the end of active bleeding and increased over the next 20 min to 59% of baseline, but never reached baseline levels (Fig. 1). Total peripheral resistance (TPR) rose in response to hemorrhage during the active hemorrhage period to 137% of baseline and remained elevated for the 3-h experimental period (141% at 180 min; Fig. 1). The survival rate and time, as well as the hemorrhage rate, are shown in Table 1. Following the uncontrolled hemorrhage, the arterial pH and base excess (BE) decreased steadily, and lactate concentration rose significantly (Table 2). There was a trend

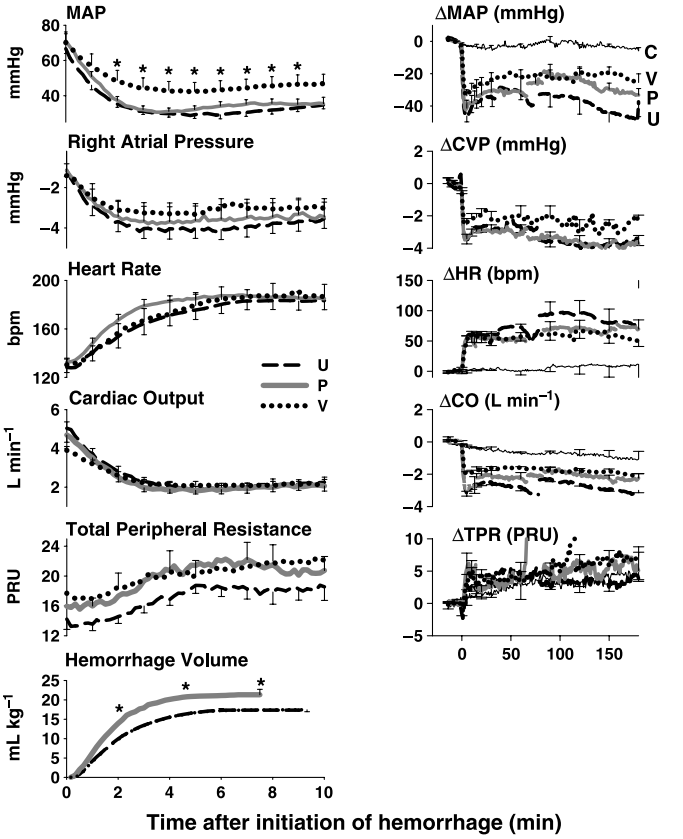

FIG. 1. **The left side of the figure shows the first 10 min of raw values for MAP, RAP, HR, CO, and TPR during the active bleeding period in the uncontrolled hemorrhage group (U, dashed line), the volume-matched controlled hemorrhage group (V, dotted line), and the pressure-matched controlled hemorrhage group (P, dark gray solid line).** The MAP in group V was significantly higher than the other two groups at the points denoted by the asterisks. The bottom panel of the left side of the figure shows the cumulative hemorrhage volume during the active phase of hemorrhage, which was complete by 6 min. The hemorrhage volume in group P was significantly higher than in groups U and V, whereas the hemorrhage volume in groups U and V were well matched. The data are the means of each group at 5-s intervals. For clarity, representative SEs are shown at various intervals. *$P < 0.05$ different from other two groups at those selected times. The right side of the figure shows the changes from baseline in the hemodynamic data averaged over 5-s intervals for each of the groups over the 3-h experimental period. For clarity, representative SEs are shown at selected intervals. The lines are discontinuous due to deaths of one or more of the animals. The ensuing data include the remaining animals.

TABLE 2. **Metabolic variances in pigs subjected to uncontrolled or controlled hemorrhage**

Time (min)	0	5	15	30	60	90	120	180
Arterial pH								
U	7.42 ± 6.01	7.47 ± 0.02*	7.46 ± 0.01	7.40 ± 0.01	7.35 ± 0.02*	7.34 ± 0.02*	7.33 ± 0.02	7.31 ± 0.03
V	7.41 ± 0.01	7.44 ± 0.01*	7.44 ± 0.01*	7.41 ± 0.01*	7.36 ± 0.01*	7.36 ± 0.01*	7.35 ± 0.02*	7.32 ± 0.03*
P	7.40 ± 0.01	7.45 ± 0.01*	7.44 ± 0.02	7.36 ± 0.01*	7.31 ± 0.02*	7.29 ± 0.02*	7.28 ± 0.02*	7.24 ± 0.02*
BEa (mM)								
U	7.1 ± 0.5	7.0 ± 0.4	5.8 ± 0.8*	2.3 ± 1.2*	−0.2 ± 2.0*	0.5 ± 1.9*	−0.5 ± 2.6	−4.0 ± 4.1
V	5.4 ± 0.9	5.8 ± 1.0*	5.1 ± 1.0*	3.3 ± 0.7*	1.0 ± 0.9*	−0.4 ± 1.2*	−1.2 ± 1.3*	−4.8 ± 2.4*
P	4.9 ± 0.7	5.2 ± 0.7	4.5 ± 0.8*	1.4 ± 1.0*	−2.0 ± 1.1*	−3.6 ± 1.3*	−5.3 ± 1.4*	−8.1 ± 1.7*
Lactate (mM)								
U	2.1 ± 0.2	2.3 ± 0.3	3.6 ± 0.6	4.5 ± 0.7*	5.7 ± 1.3*	6.0 ± 0.8*	6.3 ± 1.3*	8.8 ± 2.3*
V	2.3 ± 0.3	2.1 ± 0.2	3.0 ± 0.4	3.8 ± 0.5*	4.8 ± 0.5*	6 ± 0.8*	6.2 ± 1.2*	7.8 ± 1.8*
P	1.8 ± 0.3	2.6 ± 0.4	3.8 ± 0.8*	3.9 ± 0.7*	5.4 ± 0.9*	6.7 ± 1.1*	8.1 ± 1.7*	9.3 ± 1.0*
Hematocrit (%)								
U	31 ± 0.7	29 ± 1.6	27 ± 1.7	27 ± 1.7	28 ± 1.8	28 ± 2.9	27 ± 3.2	24 ± 1.9
V	32 ± 0.9	30 ± 0.9	30 ± 0.8	27 ± 2.5	30 ± 0.9	30 ± 0.8	29 ± 1.8	30 ± 1.2
P	32 ± 0.7	29 ± 0.7	28 ± 1.0	29 ± 1.1	29 ± 1.3	29 ± 1.5	30 ± 0.9	30 ± 0.9
Protein (g dL^{-1})								
U	5.2 ± 0.1	4.9 ± 0.2	4.7 ± 0.2	4.7 ± 0.2	4.9 ± 0.2	4.8 ± 0.2	5.0 ± 0.2	5.0 ± 0.2
V	5.7 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.6 ± 0.2	5.5 ± 0.1
P	5.6 ± 0.1	5.3 ± 0.0	5.2 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.4 ± 0.1

Data expressed as mean ± SE.
*$P < 0.05$ different from 0.

for hematocrit and plasma protein to decrease slightly during the course of the experiment, but this did not reach statistical significance (Table 2).

The site of the aortotomy was examined at necropsy. Except for the central clot (estimated volume of 10–30 mL) that lay over the aortotomy site, no retained blood was found. Thus, the time course of the weight change recorded on the balance accurately reflected the time course of the hemorrhage. Although we did not measure the aortotomy in the current study, we found in a previous study (8) that the biopsy punch made a reproducible hole such that the area of the holes among a 1.5-, 2.0- and 2.8-mm–size punch was significantly ($P < 0.05$) different (2.3 ± 0.2, 3.5 ± 0.2, and 4.7 ± 0.3 mm^2, respectively; personal observations).

Pressure-controlled hemorrhage

To match the blood pressure profile of the uncontrolled hemorrhage animals, a 17% greater blood volume was withdrawn from the group P animals (Fig. 1). The volume removed was 21.3 ± 1.4 mL kg^{-1} for group P (Table 1). To perform the match of the blood pressure profile curve in group U, the rate at which the blood had to be removed was significantly greater in group P during the first minute (Table 1; Fig. 1). The time to complete the hemorrhage was shorter in group P than in group U (Table 1). As the rate of uncontrolled hemorrhage slowed near the end of the active bleeding period, the MAP started to rise (Fig. 1). The hemorrhage was terminated at that point in the group P profile, which resulted in a shorter hemorrhage period. The decrease in CO to 41% of baseline in group P was similar to group U, as was the increase in HR to 140% of baseline (Fig. 2). This reduction in CO and increase in HR in the U and P groups were sustained throughout the experiment in both groups. The TPR significantly increased to 137% of baseline in group P in response to hemorrhage, and this increase persisted throughout the experiment (Fig. 2). There were no significant differences in arterial or venous blood gas or plasma glucose or creatinine measurements between groups (data not shown). Blood pH and BE decreased steadily, and lactate concentration rose significantly following hemorrhage, but the differences were similar in both groups U and P over the course of the experiment (Table 2). There was a trend for hematocrit and plasma protein to decrease slightly during the course of the experiment in both groups, but this did not reach statistical significance (Table 2). There was no significant difference in survival rate or time between groups U and P (Table 1).

Volume-controlled hemorrhage

The data from group V illustrate that the blood volume loss was well matched to group U (Fig. 1), and the times to completion of hemorrhage were similar in both groups (Table 1). Despite this, the lowest MAP achieved at the end of hemorrhage was 50% higher than that observed after the uncontrolled hemorrhage (Figs. 1 and 2). Group V also had significant changes in HR, CO, and TPR, which persisted through the study. When expressed as percent changes from group U, the MAP and CO were significantly higher in group V than in group U (Fig. 2). Both groups had similar changes noted in blood gases and plasma glucose and creatinine concentrations (data not shown). Blood lactate concentrations

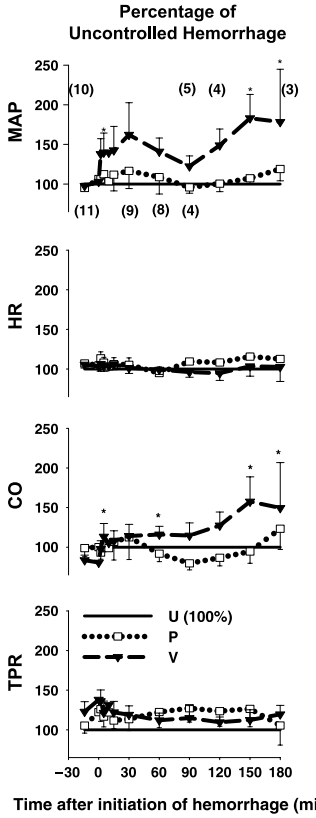

FIG. 2. **The figure shows the data expressed as the percentage of group U (black line at 100%) for selected time points (2, 5, 10, 15, 30, 60, 90, 120, 150, and 180 min).** Groups V and P started with n = 11. The numbers in parentheses above the inverted triangles are the numbers of animals alive at the specific time point for group V, and the numbers below the squares are the number of animals alive for group P. *$P < 0.05$ different from group U at the specific time point. Group V indicates black triangles; group P, white squares.

rose significantly following hemorrhage, whereas arterial pH and BE fell, but there was no statistically significant difference between groups V and U (Table 2). The reductions in plasma protein concentration and hematocrit were similar between groups U and V, suggesting equivalent rates of transcapillary refill (Table 2). There was no significant difference between group V and the other groups in survival rate or time (Table 1).

Reproducibility and variability of pressure and volume changes

This study was designed to be able to compare the P and V groups to a single U group (Figs. 2–4); therefore, we needed to have matched sets. However, in the conduct of this study, there were some animals from group P that had no matching animal from group V. To assess the reproducibility of this study, we determined if there was a similar relationship between the MAP and the volume in nonmatched animals as there were for the matched sets. The top half of Table 1 shows the data for the matched sets. The same relationship that shows a greater hemorrhage volume in the P group compared with the U and V groups is demonstrated. Likewise, the same relationship that shows a higher nadir MAP in the V group compared with the U and P groups occurred. Table 3 shows the baseline MAPs for the U groups and the number of times each animal was used to make a matched set.

Changes with hemorrhage volume

The changes from baseline of the MAP, central venous pressure (CVP), CO, HR, and TPR are shown as a function of the hemorrhage volume removed in the three groups (Fig. 3). The top panel shows the MAP as it changed in response to the hemorrhage volume. The regression equations were $U_{MAP} = -1.78 \times \text{hemvol} - 11.78$ ($r^2 = 0.994$); $P_{MAP} = -1.90 \times \text{hemvol} - 4.99$ ($r^2 = 0.979$); and $V_{MAP} = -1.45 \times \text{hemvol} - 5.85$ ($r^2 = 0.989$). The slope of the regression line for the V group was significantly less than the slope of either P or U ($P < 0.007$), whereas the slopes of P and U were not different ($P = 0.30$). The second panel shows the CVP as it changed in response to the hemorrhage volume. The regression equations were $U_{CVP} = -0.15 \times \text{hemvol} - 0.81$ ($r^2 = 0.914$); $P_{CVP} = -0.11 \times \text{hemvol} - 0.67$ ($r^2 = 0.953$); and $V_{CVP} = -0.08 \times \text{hemvol} - 0.84$ ($r^2 = 0.807$). The slope of the regression line for the P and V groups was significantly less than the slope of U ($P \leq 0.007$),

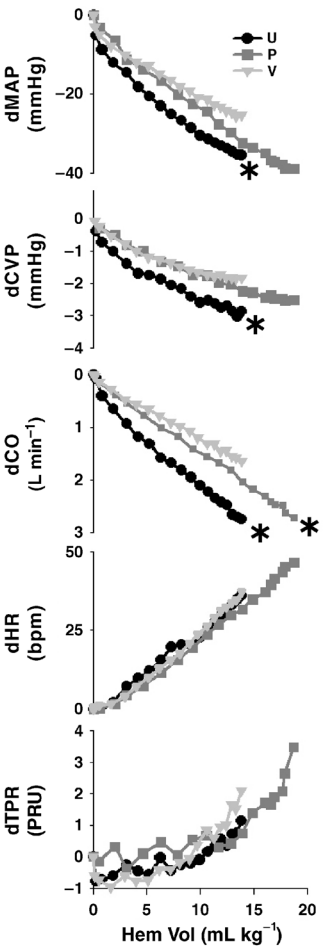

FIG. 3. **The changes from baseline of the MAP, CVP, CO, HR, and TPR during the active hemorrhage period are shown as a function of the hemorrhage volume removed.** The data are the means of each of the groups. The hemorrhage time in group P was greater than in the other two groups; therefore, the line extends further than the other two groups in each of the panels. *The group with this symbol at the end of the line is significantly different ($P < 0.05$) from the other two groups.

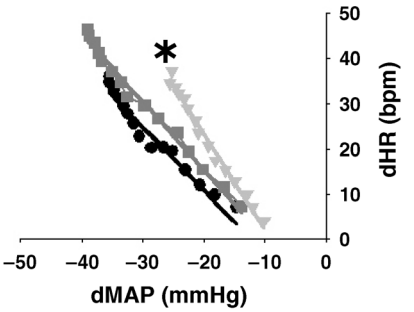

FIG. 4. **The tachycardic portion of the baroreflex curve is shown for the three groups.** The data are the means of each of the groups during the active bleeding phase. The lines are the regression lines. *The group with this symbol at the end of the line is significantly different from the other two groups. Group U indicates black circles; group P, dark gray squares; group V, light gray triangles.

and the slope of V was different from P ($P = 0.04$). The third panel shows the CO as it changed in response to the hemorrhage volume. The regression equations were $U_{CO} = -0.17 \times \text{hemvol} -0.43$ ($r^2 = 0.994$); $P_{CO} = -0.14 \times \text{hemvol} -0.11$ ($r^2 = 0.998$); and $V_{CO} = -0.11 \times \text{hemvol} -0.09$ ($r^2 = 0.997$). The slope of the regression line for the V group was significantly less than the slope of either P or U ($P < 0.001$), and the slope of P was different from U ($P < 0.001$). The fourth panel shows the HR as it changed in response to the hemorrhage volume. The regression equations were $U_{HR} = 2.82 \times \text{hemvol} -3.52$ ($r^2 = 0.881$); $P_{HR} = 2.77 \times \text{hemvol} -5.98$ ($r^2 = 0.994$); and $V_{HR} = 3.1 \times \text{hemvol} -6.35$ ($r^2 = 0.980$). The slope of the regression line for the V group was not significantly different from the slopes of either P or U ($P = 0.13$). The bottom panel shows the TPR as it changed in response to the hemorrhage volume. A quadratic equation best fit the curves (data not shown). There were no significant differences among the slopes ($P = 0.23$) or squared terms ($P = 0.09$) of the three groups.

Change in MAP versus change in HR

Figure 4 shows the tachycardic portion of the baroreflex curve of the mean response for each the three groups during the active bleeding phase. The slope of the relationship is significantly steeper in group V ($d_{HR} = -2.1 \times d_{MAP} -19$; $r^2 = 0.99$) than in groups P ($d_{HR} = -1.3 \times d_{MAP} -15$, $r^2 = 0.96$; $P = 0.02$ V vs. P) or U ($d_{HR} = -1.4 \times d_{MAP}$-17, $r^2 = 0.99$; $P = 0.05$ V vs. U).

DISCUSSION

As we hypothesized, total blood loss was greater in group P controlled-hemorrhage animals than in group U uncontrolled-hemorrhage animals, and blood pressure was 50% higher in group V controlled-hemorrhage animals compared with group U.

This study used a 2-mm skin biopsy punch to induce the aortotomy hemorrhage. The technique was used successfully in our previous study (8). Perhaps the most common aortotomy method in pigs is to induce a vascular lesion by pulling a wire, making a slit in the infrarenal aorta (2, 13). Both the biopsy punch and wire techniques have a fine line between animals that live and those that die depending on the amount of blood loss. However, the wire technique may be highly variable due to the approximation of the edges of the tear when the vessel lumen contracts as the pressure falls. Therefore, in the present study, we used the biopsy punch technique, which made a very reproducible round tissue defect, the edges of which would not reapproximate as the lumen diameter decreased, but was of a size that allowed for a clot to form.

A variety of physiological changes occur in response to hemorrhage involving hormonal, autonomic, and metabolic factors, and some of these events (e.g., tachycardia, decrease in CVP) begin even before blood pressure falls, although the mechanisms are incompletely understood (14, 15). To understand possible mechanisms associated with these differences, further analyses of the data were made. The change in hemodynamic variables with respect to hemorrhage volume over the initial bleed period is shown in Figure 3, which shows that for a given blood loss, the reduction in blood pressure was greater in the uncontrolled hemorrhage compared with either the pressure-matched or the volume-matched controlled hemorrhages. Teleologically, it can be speculated that uncontrolled hemorrhage results in an active reduction in blood pressure, thereby reducing driving pressure and minimizing blood loss. Thus, trying to reach this same blood pressure level through a controlled hemorrhage would require more blood, which is what was observed in group P. From the perspective of blood volume, further support for this speculation comes from the present observation that the same controlled blood volume loss resulted in higher MAP and smaller reduction in CO than in uncontrolled hemorrhage. Bickell et al. (2) also observed that the magnitude in the decrease in MAP as a result of an uncontrolled aortotomy hemorrhage was greater than that predicted from the estimated blood volume loss and greater than that observed following a controlled hemorrhage. However, the controlled hemorrhage study they referenced was not matched to the rate of blood loss as in the present study.

Bickell et al. (2) felt that the early hypotensive response to an uncontrolled aortotomy hemorrhage was due to the hole allowing another route for blood flow and a decrease in TPR. In their study, MAP fell significantly from baseline by 25 s, which was the earliest time they looked at the response. In the present study, the MAP was significantly less than the baseline within 10 s in groups U

TABLE 3. **Baseline MAPs of U group in the matched set and number of times used**

ID	Baseline MAP (mmHg)	No. times used/matched to P and V controlled hemorrhages
57	56	1
27	57	3
19	66	1
11	74	2
30	79	2
33	85	2

and P compared with 20 s in group V. The CO was significantly less than the baseline within 20 s in groups U and P compared with 40 s in group V. The parallel drop in MAP and CO resulted in a TPR that was constant during the time of active bleeding. Within 5 min, at the end of active bleeding, the TPR increased to a similar degree in all groups. In the present study, the CO was measured beat to beat using an ultrasonic flow probe, whereas intermittent thermodilution was used by Bickell et al. (2). They had only one measurement of CO at the end of hemorrhage at 5 min, and the TPR value was not significantly different from the baseline, although it was lower (18 mmHg $\times$ min L^{-1} at baseline to 15 mmHg $\times$ min L^{-1} at 5 min). In addition, the CO and MAP in their study had reached a nadir and then were slowly climbing. Thus, the calculated TPR was significantly reduced at 15 and 30 min following the hemorrhage. Although injection of saline for the determination of CO gives a real-time value under steady-state conditions, the thermodilution method may not be as accurate as direct measurement of CO by ultrasonic flow method under changing conditions. This difference in the methods of measuring CO and, therefore, calculated TPR may explain the discrepancy in the results between our study and theirs during the time of active bleeding and shortly thereafter.

Because TPR did not change during the time of the rapid fall in MAP in the present study, we explored possible alternative mechanisms other than arteriolar vasodilation. The greater drop in MAP in group U may be accounted for by the fact that CVP fell to a greater extent in the U group compared with group P or V as a function of hemorrhage volume. This reduced preload can be responsible for the lower CO, which can account for the lower MAP.

This greater reduction in the preload suggests that the Bezold-Jarisch (BJ) reflex may have been activated in the U group, particularly during the active bleeding period. Nganele and Hintze (16) showed that activation of coronary arterial cardiac receptors with prostaglandins or veratridine resulted in a dose-dependent reduction in preload in otherwise normal conscious dogs. They found that the reduction in preload appeared to be a result of vagally mediated withdrawal of venous sympathetic tone, and it led to a reduction of CO and MAP. If the buffering capacity of the arterial baroreceptor reflex was removed, the decreased CO response to C-fiber activation was even greater (16). A possible interpretation of the results of the current study is that the V group represents a pure arterial baroreceptor reflex-mediated compensation to a fixed volume, resulting in a defense of the MAP to the higher levels. In the group U animals, it is possible that the BJ reflex was activated by the injury, resulting in an interaction of venous tone withdrawal evidenced by the decrease in RAP and CO. This would counteract the baroreflex-mediated defense of the MAP, resulting in a reduction in MAP. In the group P animals, we can speculate that the removal of a greater volume of blood may have been required to overcome the defense of the MAP by the baroreceptor reflex, but the hemorrhage volume alone—without injury—did not result in the activation of the BJ reflex to reduce the preload, although Coleridge et al. (17) showed that cardiac deformation caused by severe hemorrhage can stimulate cardiac C-fibers. Nganele and Hintze (16) noted that a reduced preload would be advantageous to a severely injured animal because it would reduce the work and, therefore, the oxygen consumption by the heart.

Despite the lower MAP and CVP in group U compared with groups P and V, the HR response was almost identical in all three groups. When an analysis of the slopes of the relationship of the change in HR versus the change in MAP was made (Fig. 4), group U had a significantly lower slope compared with V, consistent with an attenuation of the baroreflex response in the U group (18). Whether this is due to sympathetic withdrawal, vagal stimulation, or a combination remains to be determined. Interestingly, the slope in group P was not significantly different from group U, suggesting that the rate of blood loss may influence the baroreflex response, perhaps by the BJ response (19, 20).

Early studies indicated that there is afferent input from injured tissue (21). Ballistic injury or grade V liver injury results in a larger reduction in blood pressure for a given blood loss than noted during controlled hemorrhage (9, 10). Early studies by the Coleridge laboratory established that there are vagal afferents that arise from the abdominal aorta and visceral vessels (17). Recently, it has been shown that nociceptive vagal afferents can attenuate the baroreceptor reflex (22), although the more usual finding is that nociceptive stimulation increases MAP (23). If afferent stimulation can result in attenuation of the baroreceptor response, it remains to be determined whether the small amount of tissue damaged by the 2-mm biopsy punch would provide enough stimulation of nociceptive afferents in the wall of the aorta to attenuate the baroreflex. Alternatively, the small amount of tissue damage is combined with the activation of the coagulation cascade at the site of the forming thrombus. This latter response can cause chemical stimulation of the afferents because molecules, such as those released from activated platelets (e.g., bradykinin, histamine, adenosine, and PGE2), have been shown to stimulate afferent nerves (24). Thrombin has been shown to be a potent vasodilator (25), although we found no reduction in TPR. Previous studies have shown that it also has effects on the endothelium (26), and it is a potent stimulator of platelets (27). Additional studies are needed to fully elucidate the mechanisms responsible for the hemodynamic differences between controlled and uncontrolled hemorrhage.

In summary, data from the present study illustrated some initial differences in the hemodynamic responses between controlled and uncontrolled hemorrhage. Each of the models used in the current study had their own unique features that made it unlikely to conclude that one particular model was better than any other for subsequent studies of hemorrhage and resuscitation. Our observation was that using a controlled hemorrhage model to match the MAP response of an uncontrolled hemorrhage was the hardest to reproduce. The fixed-volume hemorrhage was the easiest to reproduce and may be most appropriate to study the efficacy of different resuscitation strategies to replace volume and study the effect of whole-body ischemia. To study the aspect of trauma and

tissue injury, especially if the effect on the inflammatory and coagulation status is of interest, a model incorporating an uncontrolled hemorrhage model with tissue injury should be used. Therapeutic interventions that can be shown as beneficial in various hemorrhage models have a greater chance to transition to clinical practice than interventions studied in a single model.

ACKNOWLEDGMENTS

The authors thank Rudolph Villarreal, Johnny Nelsion, and Allean James for technical assistance and preparation of the animals, Guy Drew and Gary Muniz for the development and running of the LabView program, and Jing-Jing Wang for her expert statistical assistance.

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