Norepinephrine Decreases Fluid Requirements and Blood Loss While Preserving Intestinal Villi Microcirculation during Fluid Resuscitation of Uncontrolled Hemorrhagic Shock in Mice

Anatole Harrois, M.D, Ph.D., Nathalie Baudry, Ph.D., Olivier Huet, M.D., Ph.D., Hiromi Kato, M.D., Laurent Dupic, M.D., Manuel Lohez, B.S., Marianne Ziol, M.D., Ph.D., Eric Vicaut, M.D., Ph.D., Jacques Duranteau, M.D., Ph.D.

ABSTRACT

Background: Norepinephrine administration is controversial during hemorrhagic shock resuscitation to stabilize mean arterial pressure (MAP) level because it could have deleterious effects on local circulations. The authors investigated the effect of norepinephrine on intestinal microcirculation during fluid resuscitation in uncontrolled hemorrhagic shock.

Methods: Mice (n = 6 per group) submitted to an uncontrolled hemorrhagic shock by tail section were randomly assigned to a resuscitation with fluid but without norepinephrine to target a MAP level of 50 mmHg (FR_{50}) or 60 mmHg (FR_{60}) or a resuscitation with fluid and norepinephrine to target a MAP level of 50 mmHg ($FRNE_{50}$) or 60 mmHg ($FRNE_{60}$). Intestinal microcirculation was observed by intravital microscopy.

Results: Fluid requirements were lower in groups resuscitated with fluid and norepinephrine than in groups resuscitated with fluid without norepinephrine (74.6 ± 45.1 in FR_{50} *vs.* 28.1 ± 10.0 μl/g in $FRNE_{50}$; $P = 0.004$ and 161.9 ± 90.4 in FR_{60} *vs.* 44.5 ± 24.0 μl/g in $FRNE_{60}$; $P = 0.041$). Blood loss was not statistically different between FR_{50} and $FRNE_{50}$ (14.8 ± 8.3 *vs.* 8.5 ± 2.9 μl/g; $P = 0.180$) but was significantly lower in $FRNE_{60}$ than in FR_{60} (10.1 ± 4.2 *vs.* 22.6 ± 9.6 μl/g; $P = 0.015$). This beneficial effect was associated with the restoration of intestinal microcirculation to the same extent in fluid resuscitated groups without norepinephrine (FR_{50} and FR_{60}) and fluid resuscitated groups with norepinephrine ($FRNE_{50}$ and $FRNE_{60}$).

Conclusions: During MAP-directed resuscitation of uncontrolled hemorrhagic shock, the administration of norepinephrine decreased blood loss and fluid requirements while preserving intestinal villi microcirculation. **(Anesthesiology 2015; 122:1093-102)**

TRAUMA injury remains the leading cause of death among people aged less than 44 years old in the United States with 50% of trauma deaths ascribable to uncontrolled hemorrhagic shock in the first 24 h of care.[1] Although there is no debate about the fact that the highest priority is to control the bleeding in the presence of an uncontrolled hemorrhagic shock, some discrepancies persist concerning the initial resuscitation strategy. Fluid resuscitation may promote coagulopathy by diluting coagulation factors and favoring hypothermia.[2–4] Moreover, an excessive level of mean arterial pressure (MAP) can favor the bleeding by preventing clot formation. Therefore, subnormal hemodynamic endpoints have been recommended with target systolic blood pressure between 80 and 90 mmHg to achieve an adequate tissue perfusion with a reasonable fluid resuscitation while waiting for hemorrhage control (permissive hypotensive resuscitation).[5] The role of vasopressor agents in the initial management of traumatic hemorrhagic shock has been a matter of debate for a long time. When facing an uncontrolled hemorrhagic shock, vasopressor administration could help to reach the recommended blood pressure target and prevent hemodilution. In addition, hypotension

What We Already Know about This Topic

- Fluid resuscitation improves tissue perfusion during hemorrhagic shock, but it may promote hemorrhage by diluting coagulation factors.
- A mixture of vasopressors and fluid could lower fluid requirements; however, uncertainty persists concerning the effects of vasopressor administration on the microcirculation during hemorrhagic shock.

What This Article Tells Us That Is New

- The administration of both norepinephrine with crystalloid (normal saline) led to less fluid requirements than when animals received only normal saline to reach a target blood pressure. There was also no significant difference in the intestinal villi microcirculatory perfusion in the animals resuscitated with normal saline alone compared with animals given normal saline and norepinephrine.

Submitted for publication May 6, 2014. Accepted for publication January 1, 2015. From the Laboratoire d'Etude de la Microcirculation, Université Paris VII Lariboisère St-Louis, UMR 942, Paris, France (A.H., N.B., H.K., L.D., E.V., J.D.); Service d'Anesthésie-Réanimation Chirurgicale, Hôpital de Bicêtre, Université Paris-Sud, Hôpitaux Universitaires Paris-Sud, Assistance Publique-Hôpitaux de Paris, Le Kremlin Bicêtre, France (A.H., O.H., J.D.); Baker IDI Heart and Diabetes Institute, Monash University, Melbourne, Australia (O.H.); Service de Réanimation Pédiatrique, Hôpital Necker Enfants-Malades, Assistance Publique, Hôpitaux de Paris, Faculté de Médecine, Université Paris-Descartes, Paris, France (L.D.); Service d'Anatomie Pathologique, Groupe Hospitalier Paris-Seine-Saint Denis, Hopital Jean Verdier, AP-HP, Bondy, France (M.L., M.Z.); and Universite Paris 13, Sorbonne Paris Cite, UFR SMBH, Bobigny, France (M.L., M.Z.).

 Anesthesiology 2015; 122:1093-102

can be worsened by the sedation needed for mechanical ventilation[6] or by a vasoplegic state due to the activation of the inflammatory response to hemorrhagic shock and trauma.[7,8] Vasopressor administration could be an interesting option in this context by inducing venous adrenergic stimulation, which shifts blood from the venous unstressed volume to the systemic circulation and increases arterial pressure. Some experimental studies underlined the beneficial effect on survival of norepinephrine[9,10] and vasopressin[11] administration during resuscitation in animal models of severe uncontrolled hemorrhagic shock. However, one can argue that vasopressor agents may produce an excessive arteriolar vasoconstriction during hypovolemia with subsequent alterations of microcirculation and tissue hypoxia occurrence. Thus, the goal of the current study was to evaluate the microcirculatory effects of norepinephrine administration in a model of uncontrolled, fluid-resuscitated, hemorrhagic shock.

In this study, we hypothesized that the administration of norepinephrine during an uncontrolled hemorrhage would improve MAP, decrease fluid requirements, and improve microcirculation hemodynamics. We focused on intestinal microcirculation because it is compromised early in the course of hemorrhagic shock and has a central role in post-hemorrhagic shock multiorgan failure.[12,13]

Therefore, the aims of our study were

1. to study the effect of norepinephrine administration on fluid requirements and blood loss during uncontrolled hemorrhagic shock resuscitation and
2. to evaluate whether or not the addition of vasopressor to fluids alters intestinal mucosa microcirculation during uncontrolled hemorrhagic shock resuscitation

Materials and Methods

Animal Preparation

All procedures were approved by the institutional animal care committee: "Comité d'éthique en expérimentation animale Lariboisière-Villemin", Paris, France (authorization number: CEEALV/2012-04-02). All animals were male Balb/c mice weighing 24.9 ± 1.3 g. Animals were fed with standard mouse chow. Mice had free access to water and feeding until they were anesthetized. Anesthesia was performed by an intraperitoneal injection of 150 mg/kg ketamine (IMALGEN®; Merial, France), 5 mg/kg xylazine hydrochloride (Sigma, USA), and 1 mg/kg atropine. Anesthesia was maintained throughout the experiment with additional injections of the same drug preparation (a quarter of the initial dose). Animals were lying on a heating blanket, and temperature was continuously monitored and kept at 38°C. After anesthesia induction, a tracheostomy was performed and mice were immediately connected to a ventilator for small animals (Harvard Rodent Ventilator, model 683; Harvard Apparatus, USA) to start mechanical ventilation (tidal volume of 240 µl, respiratory rate of 80 min^{-1}, positive end expiratory pressure of 1 cm H_2O). Inspired oxygen fraction was adapted to reach a Pa_{O_2} level of 100 to 120 mmHg. The right carotid artery was cannulated with a polyethylene catheter (PE = 10, ID = 0.28) and connected to a pressure transducer linked to an acquisition system (MP-30 Biopack Systems, Goleta, CA) with real-time continuous arterial pressure monitoring. Intraarterial perfusion of Lactate Ringer solution was continuously administered at a rate of 80 µl·10 g^{-1}·h^{-1} to compensate for fluid loss. The right femoral artery was cannulated (PE = 10, ED = 0.37 mm) to perform the volume controlled hemorrhage between T_0 and T_{15}. The right femoral vein was cannulated (PE = 10, ED = 0.45 mm) to administer norepinephrine during the resuscitation phase.

Experimental Procedure

Forty-two mice were included in the study. The following model of uncontrolled hemorrhagic shock refers to the one created by Capone *et al.*[4] After surgical procedure, a stabilization period of 30 min ensued. Then, the experimental protocol was divided into three phases (fig. 1). The first step of the first phase (T_0 to T_{15}) consisted of a volume-controlled hemorrhage by withdrawing a total amount of 33 µl/g of blood (11 µl/g every 5 min). The shed blood was stored in heparinized syringes and was later used for transfusion. A stabilization period of 15 min ensued (T_{15} to T_{30}). At T_{30}, the second step of the first phase began. The mouse tail was cut at three quarters of its length from the tip until T_{90}. Blood loss from the tail was collected in a tube containing heparinized NaCl 0.9% to allow bleeding amount measurement. Between T_{30} and T_{90}, mice were resuscitated with fluid (NaCl 0.9 %), norepinephrine, or both. The 42 mice were divided into seven groups (n = 6 mice per group) according to the hemorrhage and resuscitation they were submitted to:

- A sham group: no hemorrhage, no resuscitation.
- A control group (CL): hemorrhage without resuscitation.
- Two hemorrhagic shock groups were resuscitated with fluid without norepinephrine to target a MAP of 50 or 60 mmHg (FR_{50} and FR_{60}, respectively). Fluid was administered by boli of 50 µl until MAP goal was achieved.
- A hemorrhagic shock group received norepinephrine without fluid to target a MAP level between 50 and 60 mmHg (NE group). Norepinephrine was prepared at a concentration of 100 µg/ml with an initial infusion dose of 0.02 µg·g^{-1}·h^{-1} and a maximal permitted infusion dose of 0.5 µg·g^{-1}·h^{-1}.[9]
- Two hemorrhagic shock groups were resuscitated with fluid and norepinephrine to target a MAP of 50 or 60 mmHg ($FRNE_{50}$ and $FRNE_{60}$, respectively). In these last two groups, norepinephrine infusion was increased alternatively with fluid boli administration to target the blood pressure goal. The maximal permitted infusion dose of norepinephrine was 0.5 µg·g^{-1}·h^{-1}.

The randomization took place after the surgical preparation of the animals once the intestine was exteriorized and

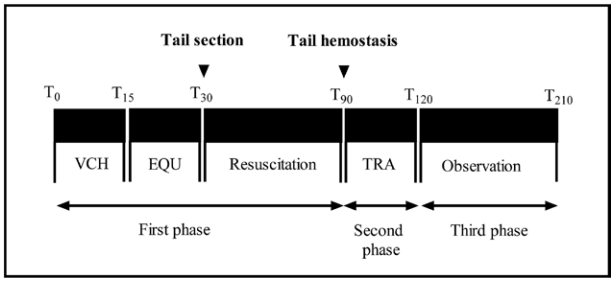

Fig. 1. Experimental protocol. EQU = equilibrium phase; TRA = retransfusion of exsanguinated blood and one volume of Lactate Ringer solution; VCH = volume controlled hemorrhage (33 ml/kg exsanguination). T_0, T_{15}, T_{30}, T_{90}, T_{120}, and T_{210} are time points at 0, 15, 30, 90, 120, and 210 min.

the catheters were inserted. After this preparation that lasted 75 min, 20% of mice were not included (the experiment was stopped) because of mucosal hemorrhagic complications or catheter insertion failure.

The first phase between T_0 and T_{90} mimicked "a prehospital phase" because transfusion and surgery were not available. At T_{90}, the second phase began: the mouse tail was cauterized to stop the uncontrolled hemorrhage. Between T_{90} and T_{120}, withdrawn blood was reinfused with an equivalent volume of Lactate Ringer solution to target a MAP of 80 mmHg and a hematocrit greater than 30%. This second phase between T_{90} and T_{120} mimicked "a hospital phase" because transfusion and surgery were available. At T_{120}, the third phase began. Mice were kept on mechanical ventilation and were observed until T_{210}. Mice were considered as survivors when they were still alive at T_{210}. Surviving animals were then sacrificed by intraarterial pentobarbital injection. Blood gases measurements were done at 0, 30, 90, and 210 min to check Pa_{O_2} level and acid-base status (Ciba Corning 248; Siemmens, Germany). Hematocrit was also measured at the end of the experiment (T_{210}).

Intravital Microscopy of Villi Microcirculation

A midline laparotomy was done to exteriorize a small segment of 2 to 3 cm of ileum. An incision was made on the antimesenteric side of the ileum greater than 1 cm. The mucosal layer was placed facing up and fastened with three pins to limit peristalsis movements. The preparation was continuously superfused with Krebs solution (in mmol/l, NaCl 118, KCl 5.9, $MgSO_4$ 0.5, NaH_2CO_3 28, $CaCl_2$ 1.25, glucose 10) heated at 37°C and bubbled with gas mixture (O_2 0%/CO_2 5%/N_2 95%). The incised ileum was covered with transparent film.

Once the surgical procedure was completed, fluorescein isothyocyanate (FITC; Sigma, USA)–labeled erythrocytes were administered intravenously at the beginning of the stabilization period. Throughout the experiment, the platform carrying mice was placed under an epifluorescence microscope (Leitz, Germany; equipped to illuminate FITC-labeled erythrocytes) to observe the intestine. Sequences of images were acquired using a charge-coupled device camera (COHU 4912; COHU Inc., USA) and were recorded on video tapes that were analyzed later by replaying the video image by image. At each measurement point, we acquired two sequences of different areas of the ileum mucosa with a 10× lens allowing for quantification of the fraction of perfused villi (defined by the number of perfused villi divided by the total number of villi observed on the field) and three different sequences of three different villi with a 25× lens to measure erythrocytes flux (corresponding to the number of erythrocytes transiting in the villous tip per unit time) and erythrocyte velocity (in the tip arteriole and in capillaries [two to five] of each studied villus).[14,15]

During the 210 min of the protocol, the experimenter could not resuscitate without knowing the MAP target, thus he was aware of the MAP objective: 50 or 60 mmHg. Norepinephrine was not administered blind, or excessive vasoconstriction would have occurred with negative consequences on the bleeding. However, the microcirculatory parameters were measured in a blinded manner (the experimenter was not aware of the group when he made the microcirculatory measurements on the video tapes).

Fluorescent Labeling of Erythrocytes

Some mice were used as erythrocytes donors. Therefore, they were anesthetized as described previously. A carotid catheter was placed to allow for exsanguination. Mice were then sacrificed by injection of sodium pentobarbital 225 mg/kg (CEVA; Santé Animale, France). Collected blood was centrifuged (5,000 rpm for 5 min) to separate erythrocytes from the plasma, and the latter were then labeled with FITC (Sigma) as previously described.[14,16] In brief, erythrocytes were washed three times in phosphate buffer saline (pH 7.4; Sigma) containing EDTA at a concentration of 100 mg/ml. Erythrocytes were then incubated in phosphate buffer saline at pH 8.0 containing FITC during 2 h at room temperature. Erythrocytes were then washed several times to eliminate FITC from the supernatant. The resulting labeled erythrocytes were stored at 4°C in darkness before their use (maximum duration of storage of 5 days).

Immunohistochemistry

The intestine was fixed in 4% paraformaldehyde immediately after mice sacrifice (T_{210}). After 48 h of fixation, the intestine sample was cut into five 3- to 5 mm-thick slices and embedded in paraffin. Dewaxed and rehydrated paraffin sections, 4-µm thick, were immunostained with the P-selectin polyclonal goat anti-mouse antibody (sc-6943; Santa-Cruz Biotechnology, USA) at a 1:20 dilution. Immunostaining was performed using an automated immunostaining device (Vision Biosystems, Australia; ER1, Menarini) for 20 min at pH 9, sections were incubated with the primary antibody for 25 min, and immunodetection was performed with a biotin-conjugated secondary rabbit anti-goat antibody (Dako, USA) followed by peroxidase-labeled streptavidin (Dako) and with diaminobenzidine chromogen as the substrate (Vision Biosystems; DAB, Menarini). Immunostained sections were

semiquantitatively scored using light microscopy. The number of P-selectin stained vessels was counted at ×20 magnification on five randomly selected fields of the mucosa or submucosa. This was repeated on four different slices.

Statistical Analysis

All results are expressed as mean ± SD except histologic results presented as median and interquartile range and results presented in figures 2 and 3 as mean ± SEM because SD would have led to unreadable figures. The effect of hemorrhage on physiologic and microcirculatory parameters was evaluated with a two-way ANOVA with one between factor: group (sham and CL) and one within factor: time (T_0, T_{30}, T_{60}, T_{90}, and T_{210}). To evaluate the effect of resuscitation on MAP, on microcirculatory parameters (erythrocyte velocity in tips and villous capillaries, erythrocytes flux in villi) and on metabolic parameters (base excess) during uncontrolled hemorrhagic shock, a two-way ANOVA analysis was conducted with one between factor: group (hemorrhagic shock groups: CL, NE, FR_{50}, FR_{60}, $FRNE_{50}$, and $FRNE_{60}$) and one within factor: time (T_{30}, T_{60}, and T_{90}) after checking that parameters were comparable at T_{30} (with a one-way ANOVA). Because the fraction of perfused villi is a non-Gaussian parameter, the effect of the different resuscitation strategies was evaluated with ANOVA on ranks. If global comparison between groups was significant, we compared all resuscitated groups with CL and compared results obtained for each target MAP in the presence or not of norepinephrine (FR_{50} *vs.* $FRNE_{50}$ and FR_{60} *vs.* $FRNE_{60}$). It should be noted that three mice died between T_{60} and T_{90} in the control group and two mice died between T_{60} and T_{90} in the norepinephrine group. That implies that n = 6 for all studied times until T_{60}. It would have been possible to consider that all microcirculatory parameters were equal to zero for the dead animals at T_{90} and T_{210}. Because considering zero value for these parameters would have increased the difference with the other groups and associated significance of the tests, we have chosen a much more conservative approach to make our conclusion more robust because we imputed the missing data (three and two dead animals in the control and the norepinephrine groups, respectively) with the average value of the group.

For fluid resuscitation requirements and blood loss, groups were compared with a Kruskal–Wallis test because the statistical distribution was non-Gaussian. If global difference among groups was significant, then for each target MAP, groups with fluid resuscitation were compared with groups with fluid resuscitation and norepinephrine (FR_{50} *vs.* $FRNE_{50}$ and FR_{60} *vs.* $FRNE_{60}$) with a Mann–Whitney test. Histologic results were analyzed with a Kruskal–Wallis test (seven groups). If global difference among groups was significant, then groups were compared two-by-two.

The statistical analysis was conducted with the software Prism (GraphPad Software, USA). Two-sided level of significance was fixed at 5% for parametric or nonparametric ANOVA. All *post hoc* comparisons were adjusted for multiplicity by Bonferroni or Dunn method for Gaussian or non-Gaussian statistical distributions, respectively.

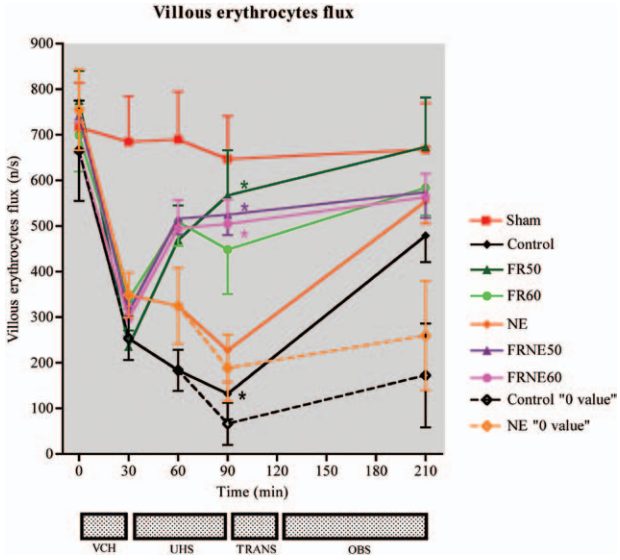

Fig. 2. Erythrocytes flux in intestinal villi in each group. *P < 0.0001 for control *vs.* sham group. *P = 0.0007, *P < 0.0001, *P < 0.0001 (FR_{50}, $FRNE_{50}$, $FRNE_{60}$ *vs.* control, respectively). P = 0.018 for FR_{60} *vs.* control group. Control group and group receiving norepinephrine without fluid (NE) were represented with the average value of the group imputed for the missing measurement points due to animal death. Additional curves with dashed lines (*i.e.*, control "0 value" and NE "0 value") correspond to the values obtained for the group when a zero value was considered for the parameter when the animal was dead at the studied time (to be more conservative no statistical tests were made with this imputation). FR_{50} and FR_{60} = groups resuscitated with fluid without norepinephrine to target a mean arterial pressure (MAP) of 50 or 60 mmHg, respectively; $FRNE_{50}$ and $FRNE_{60}$ = groups resuscitated with fluid and NE to target a MAP of 50 or 60 mmHg, respectively; NE = group receiving norepinephrine without fluid; OBS = observation phase; T_0 = baseline; T_{30} = end of the volume-controlled hemorrhage; T_{60} = during uncontrolled hemorrhagic shock; T_{90} = end of uncontrolled hemorrhagic shock; T_{210} = end of the observation phase; TRANS = transfusion; UHS = uncontrolled hemorrhagic shock; VCH = volume-controlled hemorrhage.

Results

MAP during the Experimental Protocol

Hemorrhagic shock induced a decrease in MAP across time (CL *vs.* sham; P for group × time <0.0001). MAP decreased in all hemorrhagic shock groups during the volume-controlled hemorrhagic shock stage (T_0 to T_{15}) to values ranging from 28 ± 1 to 30 ± 4 mmHg (table 1). During the stabilization period (T_{15} to T_{30}), MAP stabilized at a level ranging from 43 ± 3 to 46 ± 2 mmHg at T_{30} that was not significantly different between groups (one-way ANOVA at T_{30}; P = 0.73) (table 1). MAP was found to be significantly different between hemorrhagic shock groups (P for group × time <0.0001). During the uncontrolled hemorrhagic shock

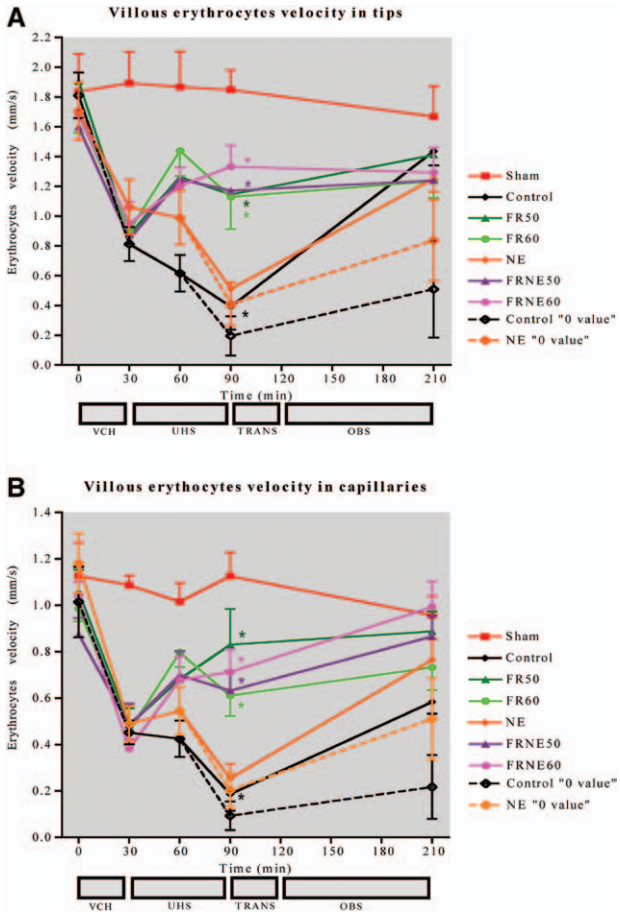

Fig. 3. Erythrocyte velocity in villous tips (*A*) and villous capillaries (*B*) in each group. *P = 0.0019, *P = 0.0003, *P = 0.0003, *P = 0.0014 (FR_{50}, FR_{60}, $FRNE_{50}$, $FRNE_{60}$ *vs.* control group [CL], respectively) for villous tip erythrocytes velocity. *P = 0.0005, *P < 0.0001 *P < 0.0001, *P = 0.0001 (FR_{50}, FR_{60}, $FRNE_{50}$, $FRNE_{60}$ *vs.* CL, respectively) for villous capillaries erythrocyte velocity. *P < 0.0001 for CL *vs.* Sham group (villous tip and villous capillaries erythrocyte velocity). CL and group receiving norepinephrine without fluid (NE) were represented with the average value of the group imputed for the missing measurement points due to animal death. Additional curves with *dashed lines* (*i.e.*, control "0 value" and NE "0 value") correspond to the values obtained for the group when a zero value was considered for the parameter when the animal was dead at the studied time (to be more conservative no statistical tests were made with this imputation). FR_{50} and FR_{60} = groups resuscitated with fluid without norepinephrine to target a mean arterial pressure (MAP) of 50 or 60 mmHg, respectively; $FRNE_{50}$ and $FRNE_{60}$ = groups resuscitated with fluid and norepinephrine to target a MAP of 50 or 60 mmHg, respectively; MAP = mean arterial pressure; NE = group receiving norepinephrine without fluid; T_0 = baseline; T_{30} = end of the volume-controlled hemorrhage; T_{60} = during uncontrolled hemorrhagic shock; T_{90} = end of uncontrolled hemorrhagic shock; T_{210} = end of the observation phase.

period (T_{30} to T_{90}), MAP was significantly higher in NE than in CL (40 ± 2 *vs.* 31 ± 6 mmHg at T_{90} in NE and CL, respectively; P = 0.01 in *post hoc* analysis). The target MAP of 50 mmHg was respected in FR_{50} and $FRNE_{50}$ groups. The target MAP of 60 mmHg was respected in $FRNE_{60}$. Although MAP decreased to 56 ± 8 mmHg at T_{90} in FR_{60}, there was no significant difference between FR_{60} and $FRNE_{60}$ (P = 0.07 in *post hoc* analysis). In hemorrhagic shock groups (CL, norepinephrine, FR_{50}, FR_{60}, $FRNE_{50}$, $FRNE_{60}$), MAP ranged from 67 ± 11 to 75 ± 14 mmHg at T_{210} (table 1).

Table 1. MAP (mmHg) and Survival (n) in Each Group

Group	T_0	T_{15}	T_{30}	T_{60}	T_{90}	T_{210}	Survival
Sham	94 ± 12	91 ± 11	90 ± 10	92 ± 10	91 ± 9	95 ± 16	6/6
Control	100 ± 9	30 ± 2	44 ± 1	38 ± 7	31 ± 6	67 ± 11*	3/6
NE	103 ± 8	29 ± 3	44 ± 3	51 ± 8	40 ± 2†	72 ± 8	4/6
FR_{50}	105 ± 12	30 ± 3	44 ± 1	52 ± 1	50 ± 2	75 ± 14	6/6
FR_{60}	94 ± 10	28 ± 1	44 ± 5	61 ± 3	56 ± 8	72 ± 10	6/6
$FRNE_{50}$	91 ± 4	29 ± 2	43 ± 3	53 ± 2	52 ± 1	68 ± 10	6/6
$FRNE_{60}$	99 ± 8	30 ± 4	46 ± 2	64 ± 3	61 ± 2	73 ± 6	6/6

*P < 0.0001 between control and sham groups across time (T_0, T_{15}, T_{30}, T_{60}, T_{90}, and T_{210}). †P = 0.01 between control and NE groups across time (T_{30}, T_{60}, and T_{90}).

FR_{50} and FR_{60} = groups resuscitated with fluid without norepinephrine to target a MAP of 50 or 60 mmHg, respectively; $FRNE_{50}$ and $FRNE_{60}$ = groups resuscitated with fluid and norepinephrine to target a MAP of 50 or 60 mmHg, respectively; MAP = mean arterial pressure; NE = group receiving norepinephrine without fluid; T_0 = baseline; T_{30} = end of the volume-controlled hemorrhage; T_{60} = during uncontrolled hemorrhagic shock; T_{90} = end of uncontrolled hemorrhagic shock; T_{210} = end of the observation phase.

The mortality in our model was 50 % because three animals out of six survived in CL. Four animals out of six survived in NE. Survival was 100% in all the other groups (table 1).

Blood Gas during the Experimental Protocol

Hemorrhagic shock led to a metabolic acidosis across time (CL *vs.* sham; P for group × time = 0.009) (table 2). No significant difference was found between metabolic acidosis that occurred in NE or CL group (CL *vs.* norepinephrine; P = 0.30). Metabolic acidosis (base excess) was not significantly different between FR_{50} (−13.2 ± 2.0 mmol/l at T_{90}) and $FRNE_{50}$ (−14.3 ± 2.0 mmol/l at T_{90}; P = 0.36) across time or between FR_{60} (−14.5 ± 2.0 mmol/l at T_{90}) and $FRNE_{60}$ (−15.2 ± 1.0 mmol/l at T_{90}; P = 0.46) across time (table 2).

Blood Loss and Fluid Requirements

Blood loss was found to be significantly different between groups (CL, NE, FR_{50}, FR_{60}, $FRNE_{50}$, $FRNE_{60}$; P = 0.01). In *post hoc* analysis, blood loss was lower in $FRNE_{60}$ group than in FR_{60} group (22.6 ± 9.6 μl/g in FR_{60} and 10.1 ± 4.2 μl/g in $FRNE_{60}$; P = 0.01). Blood loss was not significantly different between FR_{50} and $FRNE_{50}$ (14.8 ± 8.3 in FR_{50} *vs.* 8.5 ± 2.9 μl/g in $FRNE_{50}$; P = 0.17) (table 3). Fluid requirements were found to be significantly different between groups (FR_{50}, FR_{60}, $FRNE_{50}$, $FRNE_{60}$; P = 0.01). In *post hoc* analysis, fluid requirements were lower in groups resuscitated with the association of fluid and norepinephrine than in groups resuscitated with fluid without norepinephrine whether for a target MAP of 50 mmHg (74.6 ± 45.1 and 28.1 ± 10.0 μl/g in FR_{50} and $FRNE_{50}$, respectively;

Table 2. Arterial Blood Gas

Group	pH	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	Base Excess (mmol/l)	Hematocrit (%)
Sham					
T_0	7.37 ± 0.06	34 ± 6	109 ± 16	6.4 ± 3	
T_{30}	7.34 ± 0.06	36 ± 5	108 ± 9	7.3 ± 2	
T_{90}	7.31 ± 0.04	38 ± 6	105 ± 6	7.0 ± 2	
T_{210}	7.30 ± 0.02	40 ± 3	99 ± 6	8.2 ± 1	41 ± 3
Control					
T_0	7.36 ± 0.05	35 ± 6	118 ± 18	6.5 ± 4	
T_{30}	7.29 ± 0.05	32 ± 7	122 ± 18	11.7 ± 3	
T_{90}	7.22 ± 0.09	25 ± 4	121 ± 4	17.7 ± 3*	
T_{210}	7.26 ± 0.11	36 ± 5	104 ± 5	11 ± 4	38 ± 2
NE					
T_0	7.39 ± 0.08	32 ± 9	130 ± 32	6.6 ± 1	
T_{30}	7.30 ± 0.05	30 ± 9	131 ± 29	12.4 ± 3	
T_{90}	7.07 ± 0.06	40 ± 3	105 ± 8	19.3 ± 2	
T_{210}	7.24 ± 0.03	41 ± 5	99 ± 9	10.0 ± 2	36 ± 4
FR_{50}					
T_0	7.36 ± 0.03	36 ± 6	122 ± 23	5.6 ± 1	
T_{30}	7.27 ± 0.06	32 ± 7	112 ± 11	12.8 ± 2	
T_{90}	7.20 ± 0.06	40 ± 5	101 ± 13	13.2 ± 2	
T_{210}	7.25 ± 0.03	39 ± 5	105 ± 5	11.8 ± 3	36 ± 2
FR_{60}					
T_0	7.38 ± 0.02	33 ± 3	120 ± 7	6.0 ± 1	
T_{30}	7.31 ± 0.04	32 ± 2	121 ± 9	10.5 ± 2	
T_{90}	7.17 ± 0.04	40 ± 6	110 ± 6	14.5 ± 2	
T_{210}	7.21 ± 0.04	40 ± 3	108 ± 8	11.9 ± 3	37 ± 3
$FRNE_{50}$					
T_0	7.36 ± 0.04	33 ± 5	110 ± 12	7.2 ± 2	
T_{30}	7.28 ± 0.05	33 ± 6	113 ± 9	11.4 ± 1	
T_{90}	7.16 ± 0.07	43 ± 6	95 ± 15	14.3 ± 2	
T_{210}	7.27 ± 0.06	38 ± 6	102 ± 9	10.2 ± 2	37 ± 2
$FRNE_{60}$					
T_0	7.36 ± 0.05	34 ± 5	125 ± 29	6.7 ± 1	
T_{30}	7.31 ± 0.06	32 ± 6	127 ± 18	11.2 ± 2	
T_{90}	7.15 ± 0.04	41 ± 4	107 ± 14	15.2 ± 1	
T_{210}	7.29 ± 0.03	36 ± 7	106 ± 12	9.8 ± 2	36 ± 2

*P < 0.0001, base excess control *vs.* sham.

FR_{50} and FR_{60} = groups resuscitated with fluid without norepinephrine to target a MAP of 50 or 60 mmHg, respectively; $FRNE_{50}$ and $FRNE_{60}$ = groups resuscitated with fluid and norepinephrine to target a MAP of 50 or 60 mmHg, respectively. MAP = mean arterial pressure; NE = group receiving norepinephrine without fluid; $PaCO_2$ = arterial pressure of carbon dioxide; PaO_2 = arterial pressure of oxygen; T_0 = after instrumentation, before the beginning of hemorrhage; T_{30} = after volume-controlled hemorrhagic shock; T_{90} = at the end of uncontrolled hemorrhagic shock; T_{210} = at the end of the observation phase. Hematocrit analysis was done at the end of the observation phase (T_{210}).

P = 0.004) or for a target MAP of 60 mmHg (161.9 ± 90.4 and 44.5 ± 24.0 μl/g in FR_{60} and $FRNE_{60}$, respectively; P = 0.041) (table 3). There was a significant difference in NE infusion dose between groups (NE, $FRNE_{50}$, and $FRNE_{60}$) across time (time × group; P = 0.001) (table 4). The norepinephrine infusion dose was significantly higher in NE group than in $FRNE_{50}$ group (P = 0.001) and $FRNE_{60}$ group (P = 0.009). The difference between infused norepinephrine doses in $FRNE_{60}$ and $FRNE_{50}$ did not reach the statistical significance (P = 0.051).

Table 3. Fluid Requirements and Blood Loss at T_{90}

Group	Fluid Requirements, μl/g	Blood Loss, μl/g
Sham	0	0
Control	0	8.0 ± 1.1
NE	0	7.5 ± 2.0
FR_{50}	74.6 ± 45.1	14.8 ± 8.3
FR_{60}	161.9 ± 90.4	22.6 ± 9.6
$FRNE_{50}$	28.1 ± 10.0*	8.5 ± 2.9
$FRNE_{60}$	44.5 ± 24.0†	10.1 ± 4.2‡

*P = 0.004 FR_{50} *vs.* $FRNE_{50}$, †P = 0.041 FR_{60} *vs.* $FRNE_{60}$, ‡P = 0.015 FR_{60} *vs.* $FRNE_{60}$. The blood loss difference between FR_{50} and $FRNE_{50}$ did not reach statistical significance (P = 0.18).

FR_{50} and FR_{60} = groups resuscitated with fluid without norepinephrine to target a MAP of 50 or 60 mmHg, respectively; $FRNE_{50}$ and $FRNE_{60}$ = groups resuscitated with fluid and norepinephrine to target a MAP of 50 or 60 mmHg, respectively; MAP = mean arterial pressure; NE = group receiving norepinephrine without fluid.

Table 4. Norepinephrine Infusion Doses

Group	T_{30}	T_{60}	T_{90}
NE	0	0.37 ± 0.14	0.50 ± 0.00
$FRNE_{50}$	0	0.09 ± 0.03	0.28 ± 0.16*
$FRNE_{60}$	0	0.15 ± 0.07	0.43 ± 0.14†

Values are given in $\mu g \cdot g^{-1} \cdot h^{-1}$ during the uncontrolled hemorrhagic shock period (T_{30} to T_{90}) in groups receiving norepinephrine (NE, $FRNE_{50}$, and $FRNE_{60}$). Groups were compared with ANOVA for repeated measurements (T_{30}, T_{60}, and T_{90}). The administered norepinephrine dose was significantly higher in NE group than in $FRNE_{50}$ group (*P = 0.001) and $FRNE_{60}$ group (†P = 0.009). The difference between infused norepinephrine doses in $FRNE_{60}$ and $FRNE_{50}$ did not reach the statistical significance (P = 0.051).

$FRNE_{50}$ and $FRNE_{60}$ = groups resuscitated with fluid and norepinephrine to target a MAP of 50 or 60 mmHg, respectively; MAP = mean arterial pressure; NE = group receiving norepinephrine without fluid; T_{30} = end of the volume-controlled hemorrhage; T_{60} = during uncontrolled hemorrhagic shock; T_{90} = end of uncontrolled hemorrhagic shock.

Microcirculatory Parameters

Hemorrhagic shock led to a decrease in the fraction of perfused villi from 100% to 81 ± 7% across time (CL *vs.* sham; P for group × time <0.0001) (table 5). Alterations in the fraction of perfused villi observed in CL were not significantly reversed in the NE group (81 ± 13% in CL *vs.* 89 ± 4% in NE at T_{90}; P = 0.59). In fluid-resuscitated groups, the fraction of perfused villi was corrected either with fluid without norepinephrine (98 ± 2% and 100% in FR_{50} and FR_{60}; P < 0.0001 *vs.* CL) or association of fluid and norepinephrine (100% in $FRNE_{50}$ and $FRNE_{60}$; P < 0.0001 *vs.* CL). No significant difference was found between the improvement of the fraction of perfused villi in groups resuscitated with fluid alone and in groups resuscitated with fluid and norepinephrine whether for a target MAP of 50 mmHg (FR_{50} *vs.* $FRNE_{50}$; P = 0.5) or for a target MAP of 60 mmHg (FR_{60} *vs.* $FRNE_{60}$; P = 0.94).

Hemorrhagic shock decreased erythrocytes flux in villi across time (CL *vs.* sham; P for group × time <0.0001) (fig. 2). This decrease in erythrocytes flux was not significantly reversed in the NE group (132 ± 112 erythrocytes/s in CL *vs.* 227 ± 106 erythrocytes/s in norepinephrine at T_{90};

Table 5. Fraction of Perfused Villi (%) in Each Group

Group	T_0	T_{30}	T_{60}	T_{90}	T_{210}
Sham	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Control	100 ± 0	97 ± 2	95 ± 4	81 ± 13*	100 ± 0
NE	100 ± 0	95 ± 3	95 ± 4	89 ± 4	100 ± 0
FR_{50}	100 ± 0	93 ± 4	98 ± 2	100 ± 0†	100 ± 0
FR_{60}	100 ± 0	93 ± 4	100 ± 0	100 ± 0†	100 ± 0
$FRNE_{50}$	100 ± 0	94 ± 6	100 ± 0	100 ± 0†	100 ± 0
$FRNE_{60}$	100 ± 0	94 ± 4	100 ± 0	100 ± 0†	99 ± 1

*P < 0.0001 control *vs.* sham, †P < 0.0001 (FR_{50}, FR_{60}, $FRNE_{50}$, and $FRNE_{60}$) *vs.* control.

FR_{50} and FR_{60} = groups resuscitated with fluid without norepinephrine to target a MAP of 50 or 60 mmHg, respectively; $FRNE_{50}$ and $FRNE_{60}$ = groups resuscitated with fluid and norepinephrine to target a MAP of 50 or 60 mmHg, respectively; MAP = mean arterial pressure; NE = group receiving norepinephrine without fluid; T_0 = after instrumentation, before hemorrhage; T_{30} = after volume-controlled hemorrhagic shock; T_{60} = during uncontrolled hemorrhagic shock; T_{90} = at the end of uncontrolled hemorrhagic shock; T_{210} = at the end of the observation phase.

Table 6. Intestine Immunostaining for P-Selectins

Groups						
Sham	Control	FR_{50}	FR_{60}*	$FRNE_{50}$	$FRNE_{60}$	NE
2 (2,3)	3 (2,3)	3 (2,3)	6 (5–7)	2 (1–3)	2 (2–4)	3 (2–4)

Values are represented as median number of stained vessels per intestine section (interquartile range).

*P < 0.01 FR_{60} *vs.* the other groups.

FR_{50} and FR_{60} = groups resuscitated with fluid without norepinephrine to target a MAP of 50 or 60 mmHg, respectively. $FRNE_{50}$ and $FRNE_{60}$ = groups resuscitated with fluid and norepinephrine to target a MAP of 50 or 60 mmHg, respectively; MAP = mean arterial pressure; NE = group receiving norepinephrine without fluid.

P = 0.72). Erythrocytes flux was significantly improved in resuscitated groups either with fluid (FR_{50} *vs.* CL; P = 0.0007 and FR_{60} *vs.* CL; P = 0.018) or the association of fluid and norepinephrine ($FRNE_{50}$ *vs.* CL; P < 0.0001 and $FRNE_{60}$ *vs.* CL; P < 0.0001) but did not return to baseline level (sham group). No significant difference was found between the improvement of the erythrocytes flux in groups resuscitated with fluid and in those resuscitated with fluid and norepinephrine whether for a target MAP of 50 mmHg (FR_{50} *vs.* $FRNE_{50}$; P = 0.48) or for a target MAP of 60 mmHg (FR_{60} *vs.* $FRNE_{60}$; P = 0.52).

Hemorrhagic shock decreased erythrocyte velocity in villous tips (CL *vs.* sham; P for group × time <0.0001) and in villous capillaries across time (CL *vs.* sham; P for group × time <0.0001) (fig. 3). This decrease in erythrocyte velocity was not significantly corrected in the NE group either in tips (0.39 ± 0.39 mm/s in CL *vs.* 0.51 ± 0.29 mm/s in NE at T_{90}; P = 0.79) or in capillaries (0.19 ± 0.17 mm/s in CL *vs.* 0.25 ± 0.15 mm/s in NE at T_{90}; P = 0.70). Erythrocyte velocity in tips was significantly improved in resuscitated groups either with fluid (FR_{50} *vs.* CL; P = 0.0019 and FR_{60} *vs.* CL; P = 0.0003) or the association of fluid and norepinephrine ($FRNE_{50}$ *vs.* CL; P = 0.0003 and $FRNE_{60}$ *vs.* CL; P = 0.0014) but did not return to baseline level (sham group). No significant difference was found between the improvement of the tips erythrocyte velocity in groups resuscitated with fluid or in groups resuscitated with fluid and norepinephrine (FR_{50} *vs.* $FRNE_{50}$; P = 0.96 and FR_{60} *vs.* $FRNE_{60}$; P = 0.16). Erythrocyte velocity in capillaries was significantly improved in groups resuscitated with fluid (FR_{50} *vs.* CL; P = 0.0005 and FR_{60} *vs.* CL; P < 0.0001) and in groups resuscitated with the association of fluid and norepinephrine ($FRNE_{50}$ *vs.* CL; P < 0.0001 and $FRNE_{60}$ *vs.* CL; P < 0.0001) but did not return to baseline level (sham group). No significant difference was found between the improvement in capillaries erythrocyte velocity in groups resuscitated with fluid and in groups resuscitated with fluid and norepinephrine whether for a target MAP of 50 mmHg (FR_{50} *vs.* $FRNE_{50}$; P = 0.33) or for a target MAP of 60 mmHg (FR_{60} *vs.* $FRNE_{60}$; P = 0.20).

Immunohistochemistry

P-selectin expression was observed in endothelial cells of the submucosal and villi vessels. P-selectin stained vessels were present in the sham group (table 6). The number of P-selectin stained vessels per intestine slice was found to be significantly different between groups (P < 0.0001). In *post hoc* analysis, the number of P-selectin stained vessels per intestine slice was not significantly increased in the CL group compared with than in the sham group (table 6). In resuscitated groups, the level of P-selectin immunostaining increased significantly in FR_{60} group compared with that in all the other groups (P < 0.01) (table 6 and fig. 4).

Discussion

In this study, we report the following observations: (1) uncontrolled hemorrhagic shock led to intestinal villi hypoperfusion with alterations in the fraction of perfused villi, decreased villous erythrocytes flux, and decreased villous erythrocyte velocity; (2) a MAP-directed resuscitation associating fluid and norepinephrine decreased blood loss and fluid requirements in comparison with a MAP-directed resuscitation with fluid resuscitation alone; (3) the addition of norepinephrine to fluid resuscitation does not produce deleterious effects on intestinal villi perfusion during uncontrolled hemorrhagic shock.

Due to its pharmacological properties, norepinephrine administration has several hemodynamic effects: (1) it increases venous return due to its venoconstrictive effect[17]; (2) it increases cardiac inotropism due to its β-agonist effect[18]; and (3) it increases arterial vasomotor tone by α-agonist effect. Because of the third effect, norepinephrine administration may decrease tissue perfusion, and more specifically in hypovolemia and during hemorrhagic shock.[19,20] Indeed, experimental models of hemorrhagic shock reported a deleterious effect of norepinephrine administration on microcirculation.[21–25] However, in these studies, animals were not fluid-resuscitated when norepinephrine was administered. During hemorrhagic shock, a vasoconstriction of A1 (70 to 120 μm) and A2 intestinal arterioles (40 to 60 μm) rapidly occurs[26,27] with a subsequent decrease in downstream blood

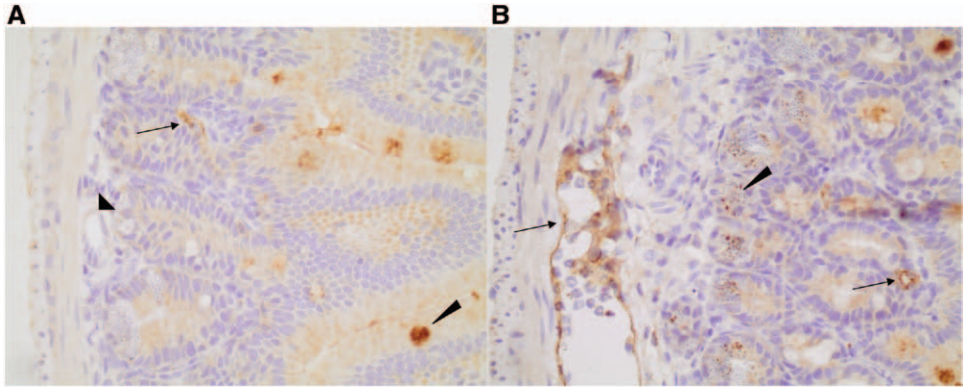

Fig. 4. P-selectin immunostaining in control group (*A*) and FR_{60} group (*B*). In control group, most vessels in the submucosa and mucosa were negative for P-selectin (*big arrowhead*). Rare vessels showed a slight positivity for P-selectin (*arrow*) (*A*). In FR_{60}, most vessels in the submucosa (*arrow*) and mucosa (*arrow*) showed a strong and continuous P-selectin staining (*B*). Brown staining in granules and in mucus of goblet cells (*thin arrowheads*) is a nonspecific staining.

flow.[28,29] As a result, norepinephrine administration without correction of hypovolemia may further increase arteriolar vasoconstriction and decrease microcirculatory blood flow. The effects of norepinephrine during hemorrhagic shock resuscitation should therefore be studied with simultaneous fluid resuscitation.

To investigate experimentally the effect of norepinephrine in a condition similar to the initial phase of hemorrhagic shock when hemorrhage is still active (uncontrolled hemorrhagic shock), Poloujadoff *et al.*[9] conducted an elegant study where rats were subjected to a MAP-directed fluid resuscitation either with or without norepinephrine administration. In this study, authors reported increased rat survival after fluid resuscitation with norepinephrine compared with a strategy based on fluid resuscitation alone. Similar results were observed in experimental uncontrolled hemorrhagic shock with liver trauma.[11,30] Although vasopressor use increased survival in these models, no information was provided on tissue perfusion, including intestinal microcirculation behavior during hemorrhagic shock.

In our model, uncontrolled hemorrhage induced a deep and sustained decrease in intestinal microcirculatory perfusion (decreased fraction of perfused villi, decreased villous erythrocytes flux, and decreased erythrocyte velocity) with an early mortality of 50% (CL). A resuscitation strategy with norepinephrine without fluid resuscitation (NE) failed to improve intestinal microcirculatory perfusion, showing persistent intestinal microcirculatory hypoperfusion comparable with the CL group (hemorrhagic shock, no fluid resuscitation). However, a resuscitation strategy using fluid resuscitation in association with norepinephrine improved intestinal microcirculatory perfusion. Although intestinal mucosa perfusion did not return to basal level (sham group), it improved to the same extent as when a resuscitation strategy with fluid resuscitation but no norepinephrine is implemented.

Fluid resuscitation remains the cornerstone of hemorrhagic shock resuscitation in an attempt to maintain tissue perfusion while waiting for rapid surgical or radiological control of the bleeding. However, given in an excessive amount, fluid resuscitation may favorize bleeding[2,31] because of hemodilution that weakens clot formation[32] and potentially because of MAP elevation that may remove the clot.[33] In our study, blood loss was lower in resuscitation strategies involving fluid and norepinephrine than in strategies involving fluid without norepinephrine. Our hypothesis is that norepinephrine avoids deleterious hemodilution, which results in less blood loss. However, coagulation tests would have been necessary to confirm this hypothesis. Several experimental studies conducted on uncontrolled hemorrhagic shock models demonstrated a beneficial effect on survival of a reasonable amount of fluid resuscitation[3,34] compared with resuscitation strategies with excessive fluid administration. Indeed, partial restoration of volemia with fluid resuscitation not only restored splanchnic perfusion[35] but also corrected oxygen debt[34] during experimental hemorrhage (while excessive fluid resuscitation corrected hemodynamic parameters but worsened microcirculation[35]). Excessive fluid resuscitation was also reported to favorize apoptosis in a model of uncontrolled hemorrhagic shock by tail section in rats.[36] In the current study, in fluid resuscitated groups, a high amount of fluid (FR_{60}) led to the overexpression of intestine endothelial P-selectin, an adhesion molecule that enhances the adhesion of leucocytes to the endothelium with subsequent tissue inflammation. The co-administration of norepinephrine with fluid during resuscitation not only respected mucosal intestinal perfusion but also decreased fluid needs to achieve a MAP level of either 50 or 60 mmHg and prevented P-selectin overexpression.

At the end of the experiment (T_{210}), the microcirculatory perfusion is similar in all seven groups (figs. 2 and 3). However, we cannot conclude that the different strategies have similar effects because 50% and 33% of mice died in the control group and in the NE group, respectively. Indeed, the microcirculatory parameters are only represented on the two figures (figs. 2 and 3) for surviving animals. It would

have been possible to consider that all microcirculatory parameters were equal to zero at T_{90} and T_{210} for the three and two dead animals in the control and in the NE group, respectively. However, we have chosen a much more conservative approach on the graphic representation. To take into account those animals that died during uncontrolled hemorrhagic shock, we added two curves on figures 2 and 3: a curve representing the whole control group (with value "0" for the dead animals) and a curve representing the whole NE group (with value "0" for the dead animals). Concerning the immunochemistry analysis, the FR_{60} group expressed more adhesion proteins in intestinal vessels demonstrating a strongest activation of the endothelium in this group. Thus, a strategy requiring excessive fluid resuscitation (FR_{60}) is not equivalent to a strategy needing less fluid (FR_{50}, $FRNE_{50}$, and $FRNE_{60}$). A longer observation phase could have shown differences of tissue perfusion between groups, but it would need animal awakening that cannot be done because intestine reparation is not possible.

There are several limitations to our study. 1) Late mortality of the mice could not be evaluated because of the invasiveness of the intestinal microcirculation analysis, which prevented the awakening of animals after the procedure. The mortality in our model (50% in CL) was less than reported by teams working on the same uncontrolled hemorrhagic shock models in rats (Capone *et al.*[4]: 50% mortality at T_{90}, 90% mortality at 72 h; Poloujadoff *et al.*[9]: 90% at T_{210}; Lu *et al.*[36]: 62,5 % mortality at T_{150}). 2) We evaluated metabolic acidosis with the calculated base excess. The measurement of serum lactate could have been an additional interesting index to evaluate the metabolic consequences of tissue hypoperfusion. However, increasing blood withdrawal at each measurement point would have led to excessive blood spoliation in small animals. 3) We conducted our experiment on mice who could behave differently from rats. Moreover, the mice were mechanically ventilated from the beginning of the protocol because intestinal microcirculation study requires a laparotomy in lateral decubitus that interferes with spontaneous ventilation. Because mechanical ventilatory support insures a stable oxygenation and decreases mice respiratory work, it may have increased survival compared with a similar model with spontaneous breathing. 4) The complex experimental model used in the current study led us to a sample size of n = 6 per group. This rather small sample size allows large effect-size evaluation. However, it may not allow a sufficient power to detect some differences in treatments associated to intermediate effect-size. 5) While administering norepinephrine, we increased the dose according to the target MAP rather than using a predetermined dose of norepinephrine that could have led to the overcorrection of the MAP.[9] The resulting hypertension could have induced adverse effects on clot stability at the injury site. Thus, resuscitation was conducted with a regular increase in norepinephrine administration alternating with a fluid resuscitation bolus, thereby insuring a well-balanced resuscitation. With this calibrated strategy, norepinephrine did not alter intestinal mucosa perfusion in our model. Such balanced resuscitation is easily managed experimentally but may be difficult to apply in the field.

Conclusions

In a mice model of uncontrolled hemorrhagic shock, a MAP-directed resuscitation associating norepinephrine and fluid resuscitation decreased blood loss and fluid requirements compared with a MAP-directed resuscitation with fluid without norepinephrine, while preserving intestinal villi microcirculation.

Acknowledgments

This work was supported by a grant from the French Society of Anesthesiology (Société Française d'Anesthésie-Réanimation, Paris, France).

Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Duranteau: Service d'Anesthésie-Réanimation Chirurgicale, Hôpital de Bicêtre, Université Paris-Sud, Hôpitaux Universitaires Paris-Sud, Assistance Publique-Hôpitaux de Paris, Le Kremlin Bicêtre, France. jacques.duranteau@bct.aphp.fr. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

References

1. Kauvar DS, Lefering R, Wade CE: Impact of hemorrhage on trauma outcome: An overview of epidemiology, clinical presentations, and therapeutic considerations. J Trauma 2006; 60(6 Suppl):S3–11
2. Abu-Hatoum O, Bashenko Y, Hirsh M, Krausz MM: Continuous fluid resuscitation and splenectomy for treatment of uncontrolled hemorrhagic shock after massive splenic injury. J Trauma 2002; 52:253–8
3. Burris D, Rhee P, Kaufmann C, Pikoulis E, Austin B, Eror A, DeBraux S, Guzzi L, Leppäniemi A: Controlled resuscitation for uncontrolled hemorrhagic shock. J Trauma 1999; 46:216–23
4. Capone AC, Safar P, Stezoski W, Tisherman S, Peitzman AB: Improved outcome with fluid restriction in treatment of uncontrolled hemorrhagic shock. J Am Coll Surg 1995; 180:49–56
5. Spahn DR, Bouillon B, Cerny V, Coats TJ, Duranteau J, Fernández-Mondéjar E, Filipescu D, Hunt BJ, Komadina R, Nardi G, Neugebauer E, Ozier Y, Riddez L, Schultz A, Vincent JL, Rossaint R: Management of bleeding and coagulopathy following major trauma: An updated European guideline. Crit Care 2013; 17:R76
6. Vatner SF, Braunwald E: Cardiovascular control mechanisms in the conscious state. N Engl J Med 1975; 293:970–6
7. Dalibon N, Schlumberger S, Saada M, Fischler M, Riou B: Haemodynamic assessment of hypovolaemia under general anaesthesia in pigs submitted to graded haemorrhage and retransfusion. Br J Anaesth 1999; 82:97–103
8. Song R, Bian H, Wang X, Huang X, Zhao KS: Mitochondrial injury underlies hyporeactivity of arterial smooth muscle in severe shock. Am J Hypertens 2011; 24:45–51

9. Poloujadoff MP, Borron SW, Amathieu R, Favret F, Camara MS, Lapostolle F, Vicaut E, Adnet F: Improved survival after resuscitation with norepinephrine in a murine model of uncontrolled hemorrhagic shock. Anesthesiology 2007; 107:591–6
10. Lee JH, Kim K, Jo YH, Kim KS, Lee CC, Kwon WY, Rhee JE, Suh GJ, Singer AJ: Early norepinephrine infusion delays cardiac arrest after hemorrhagic shock in rats. J Emerg Med 2009; 37:376–82
11. Voelckel WG, Raedler C, Wenzel V, Lindner KH, Krismer AC, Schmittinger CA, Herff H, Rheinberger K, Königsrainer A: Arginine vasopressin, but not epinephrine, improves survival in uncontrolled hemorrhagic shock after liver trauma in pigs. Crit Care Med 2003; 31:1160–5
12. Leaphart CL, Tepas JJ 3rd: The gut is a motor of organ system dysfunction. Surgery 2007; 141:563–9
13. Reino DC, Pisarenko V, Palange D, Doucet D, Bonitz RP, Lu Q, Colorado I, Sheth SU, Chandler B, Kannan KB, Ramanathan M, Xu da Z, Deitch EA, Feinman R: Trauma hemorrhagic shock-induced lung injury involves a gut-lymph-induced TLR4 pathway in mice. PLoS One 2011; 6:e14829
14. Nakajima Y, Baudry N, Duranteau J, Vicaut E: Microcirculation in intestinal villi: A comparison between hemorrhagic and endotoxin shock. Am J Respir Crit Care Med 2001; 164(8 Pt 1):1526–30
15. Sarelius IH, Duling BR: Direct measurement of microvessel hematocrit, red cell flux, velocity, and transit time. Am J Physiol 1982; 243:H1018–26
16. Butcher EC, Weissman IL: Direct fluorescent labeling of cells with fluorescein or rhodamine isothiocyanate. I. Technical aspects. J Immunol Methods 1980; 37:97–108
17. Nouira S, Elatrous S, Dimassi S, Besbes L, Boukef R, Mohamed B, Abroug F: Effects of norepinephrine on static and dynamic preload indicators in experimental hemorrhagic shock. Crit Care Med 2005; 33:2339–43
18. Hamzaoui O, Georger JF, Monnet X, Ksouri H, Maizel J, Richard C, Teboul JL: Early administration of norepinephrine increases cardiac preload and cardiac output in septic patients with life-threatening hypotension. Crit Care 2010; 14:R142
19. Sperry JL, Minei JP, Frankel HL, West MA, Harbrecht BG, Moore EE, Maier RV, Nirula R: Early use of vasopressors after injury: Caution before constriction. J Trauma 2008; 64:9–14
20. Plurad DS, Talving P, Lam L, Inaba K, Green D, Demetriades D: Early vasopressor use in critical injury is associated with mortality independent from volume status. J Trauma 2011; 71:565–70; discussion 570–2
21. Hershey SG, Mazzia VD, Altura BM, Gyure L: Effects of vasopressors on the microcirculation and on survival in hemorrhagic shock. Anesthesiology 1965; 26:179–89
22. Jackson AJ, Webb WR: Effect of norepinephrine oon differential blood flow in graded hemorrhagel. Surg Forum 1962; 13:14–6
23. Vowles KD, Barse FE, Bovard WJ, Couves CM, Howard JM: Studies of coronary and peripheral blood flow following hemorrhagic shock, transfusion and L-norepinephrine. Ann Surg 1961; 153:202–8
24. Schumer W: Study of the effects of norepinephrine on the microcirculation of the dog omentum in oligemic shock. Surg Forum 1963; 14:19–21
25. Torrance HB: The effect of the intravenous infusion of noradrenaline on splanchnic blood flow in haemorrhagic shock. Clin Sci 1961; 20:401–6
26. Cryer HM, Gosche J, Harbrecht J, Anigian G, Garrison N: The effect of hypertonic saline resuscitation on responses to severe hemorrhagic shock by the skeletal muscle, intestinal, and renal microcirculation systems: Seeing is believing. Am J Surg 2005; 190:305–13
27. Fruchterman TM, Spain DA, Wilson MA, Harris PD, Garrison RN: Complement inhibition prevents gut ischemia and endothelial cell dysfunction after hemorrhage/resuscitation. Surgery 1998; 124:782–91; discussion 791–2
28. Vajda K, Szabó A, Boros M: Heterogeneous microcirculation in the rat small intestine during hemorrhagic shock: Quantification of the effects of hypertonic-hyperoncotic resuscitation. Eur Surg Res 2004; 36:338–44
29. Dubin A, Pozo MO, Ferrara G, Murias G, Martins E, Canullán C, Canales HS, Kanoore Edul VS, Estenssoro E, Ince C: Systemic and microcirculatory responses to progressive hemorrhage. Intensive Care Med 2009; 35:556–64
30. Stadlbauer KH, Wagner-Berger HG, Raedler C, Voelckel WG, Wenzel V, Krismer AC, Klima G, Rheinberger K, Nussbaumer W, Pressmar D, Lindner KH, Königsrainer A: Vasopressin, but not fluid resuscitation, enhances survival in a liver trauma model with uncontrolled and otherwise lethal hemorrhagic shock in pigs. Anesthesiology 2003; 98:699–704
31. Solomonov E, Hirsh M, Yahiya A, Krausz MM: The effect of vigorous fluid resuscitation in uncontrolled hemorrhagic shock after massive splenic injury. Crit Care Med 2000; 28:749–54
32. Marshall HP Jr, Capone A, Courcoulas AP, Harbrecht BG, Billiar TR, Udekwu AO, Peitzman AB: Effects of hemodilution on long-term survival in an uncontrolled hemorrhagic shock model in rats. J Trauma 1997; 43:673–9
33. Sondeen JL, Coppes VG, Holcomb JB: Blood pressure at which rebleeding occurs after resuscitation in swine with aortic injury. J Trauma 2003; 54(5 Suppl):S110–7
34. Siegel JH, Fabian M, Smith JA, Kingston EP, Steele KA, Wells MR, Kaplan LJ: Oxygen debt criteria quantify the effectiveness of early partial resuscitation after hypovolemic hemorrhagic shock. J Trauma 2003; 54:862–80; discussion 880
35. Varela JE, Cohn SM, Diaz I, Giannotti GD, Proctor KG: Splanchnic perfusion during delayed, hypotensive, or aggressive fluid resuscitation from uncontrolled hemorrhage. Shock 2003; 20:476–80
36. Lu YQ, Cai XJ, Gu LH, Wang Q, Huang WD, Bao DG: Experimental study of controlled fluid resuscitation in the treatment of severe and uncontrolled hemorrhagic shock. J Trauma 2007; 63:798–804