Microcirculatory Parameters After Isotonic and Hypertonic Colloidal Fluid Resuscitation in Acute Hemorrhagic Shock

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Background: **Volume resuscitation is one of the primary therapeutic goals in hemorrhagic shock, but data on microcirculatory effects of different colloidal fluid resuscitation regimen are sparse. We investigated sublingual mucosal microcirculatory parameters during hemorrhage and after fluid resuscitation with gelatin, hydroxyethyl starch, or hypertonic saline and hydroxyethyl starch in pigs.**

Methods: **To induce hemorrhagic shock, 60% of calculated blood volume was withdrawn. Microvascular blood flow was assessed by laser Doppler velocimetry. Microcirculatory hemoglobin oxygen saturation was measured with a tissue reflectance spectrophotometry, and side darkfield imaging was used to visualize the microcirculation and to quantify the flow quality. Systemic hemodynamic variables, systemic acid base and blood gas variables, and lactate measurements were recorded. Measurements were performed at baseline, after hemorrhage, and after fluid resuscitation with a fixed volume regimen.**

Results: **Systemic hemodynamic parameters returned or even exceeded to baseline values in all three groups after fluid resuscitation, but showed significantly higher filling pressures and cardiac output values in animals treated with isotonic colloids. Microcirculatory parameters determined in gelatin and hydroxyethyl starch resuscitated animals, and almost all parameters except microvascular hemoglobin oxygen saturation in animals treated with hypertonic saline and hydroxyethyl starch, were restored after treatment.**

Discussion: **Hemorrhaged pigs can be hemodynamically stabilized with either isotonic or hypertonic colloidal fluids. The main finding is an adequate restoration of sublingual microcirculatory blood flow and flow quality in all three study groups, but only gelatin and hydroxyethyl starch improved microvascular hemoglobin oxygen saturation, indicating some inadequate oxygen supply/demand ratio maybe due to a better restoration of systemic hemodynamics in isotonic colloidal resuscitated animals.**

Key Words: **Gelatine, Hydroxyethylstarch, Hypertonic saline, Side dark field imaging, Laser Doppler flowmetry, Tissue reflectance spectrophotometry.**

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Resuscitation of the trauma patient with hemorrhagic shock requires the identification of the bleeding source followed by prompt prevention of further blood loss. After bleeding, control fluid resuscitation is initiated to achieve hemodynamic stability to restore tissue perfusion and tissue oxygen supply.

Although several meta-analysis have shown an increased risk of death in patients treated with colloids compared with patients treated with crystalloids,[1–4] colloidal fluid resuscitation after bleeding control may be beneficial in the face of tissue oxygen delivery.[5] But, there is not only intense discussion regarding colloids versus crystalloids, but also the debate which colloidal fluid is superior to another.[6,7] Furthermore, promising results have been obtained with hypertonic solutions.[8] The so-called small volume resuscitation of the systemic circulation ensures restoration of regional blood flow to vital organs.[9–11] Also the amount of fluid volume to be given to substitute the blood loss is still under debate. Recommendations exists to substitute blood 1:1 with hydroxyethyl starch (HES), 1:1.3 with gelatin, and to administer 4 mL/kg hypertonic saline/HES (HS-HES) to restore systemic hemodynamics.[12,13] Furthermore, a target Hb of 7 to 9 g/dL is recommended.[14]

Microcirculatory dysfunction has been hypothesized to play a key role in the pathophysiology of organ failure and consequently patient outcome, as the supply of oxygen takes place at the microcirculatory level.[15,16] Furthermore, inadequate or to late resuscitation of patients suffering from hemorrhagic shock consequently leads to the syndrome of multiple organ dysfunction.[17] Although volume resuscitation is one of the primary therapeutic goals in hemorrhagic shock, data on effects of different fluid resuscitation regimen on microcirculatory blood flow and tissue oxygen supply are sparse, and results of studies are heterogeneous.[5,18–22]

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Hypertonic solutions have been demonstrated to bring about rapid improvements in both, the macro- and the microhemodynamics,[9,21–23] but direct comparisons with other colloids are sparse.

For that reason the relationship between different kind of fluid resuscitation and microcirculatory parameters was investigated by side darkfield imaging, laser Doppler velocimetry, and tissue reflectance spectrophotometry at the sublingual mucosa. It was hypothesized that there is an association between the kind of fluid used and differences in systemic hemodynamics and microcirculatory parameters in a hemorrhagic shock pig model.

MATERIALS AND METHODS

The study was approved by the Austrian Federal Animal Investigation Committee, and the animals were managed in accordance with the American Physiologic Society institutional guidelines and the Position of the American Heart Association on Research Animal Use, as adopted on November 11, 1984. Animal care was performed by qualified individuals supervised by veterinarians, and all the facilities and transportation comply with current legal requirements and guidelines. Anesthesia was used in all surgical interventions, all unnecessary suffering was avoided. Our animal facilities meet the standards of the American Association for Accreditation of Laboratory Animal Care. The present study was performed in collaboration with another study group investigating coagulation alterations in the same animals at the same time to keep the number of research animals as low as possible, according to recommendation of the Austrian Federal Animal Investigation Committee.

Anesthesia and Animal Instrumentation

Thirty domestic pigs (35–45 kg) were made to fast for 12 hours, but had free access to water. One hour before surgery, pigs were premedicated with azaperone (4 mg/kg i.m., Stresnil, Janssen, Vienna, Austria) and 0.1 mg/kg atropine i.m. (Atropinum sulfuricum, Nycomed, Linz, Austria). Anesthesia was induced with 20 mg/kg ketamine i.m. (Ketaminol, Intervet, Vienna, Austria) and intravenous administration of propofol (1–2 mg/kg, Diprivan 1%, Abott, Vienna, Austria) and maintained with propofol ($6-8\ mg \cdot kg^{-1} \cdot h^{-1}$ i.v.). Analgesia was performed with piritramid (30 mg i.v., Dipidolor, Janssen, Vienna, Austria). Animals were intubated and mechanically ventilated with a positive end-expiratory pressure of 5 mm Hg. Tidal volume and respiratory frequency were adjusted to maintain a pCO_2 of 35 to 45 mm Hg at baseline; fractional inspiratory oxygen concentration was set at 0.35. A 6-Fr catheter was inserted into the femoral artery for collection of blood samples and continuous arterial pressure monitoring. A 12-Fr large bore catheter was advanced into the femoral vein for blood withdrawing and volume resuscitation. After preparation of the internal jugular vein, a 7.5-Fr pulmonary artery catheter (Baxter, Irvine, CA) was inserted. The baseline fluid requirement ($4\ mL \cdot kg^{-1} \cdot h^{-1}$ i.v.) was substituted with crystalloid (Ringer's lactate) via a peripheral venous access during the entire course of the procedure. Body temperature was maintained between 38.0° and 39.0°C.

Hemodynamic and Blood Gas Measurements

Arterial-, pulmonary artery, and central venous pressure were measured using three Statham P10EZ pressure transducers (Spectramed-Statham, Bilthoven, Netherlands). Cardiac output was determined using the thermodilution method. Heart rate, blood pressure, and core temperature were continuously recorded. Zero reference for all pressures was the mid-chest position. Arterial blood gases and acid-base status were determined using an automatic blood gas analyzer (AVL 995, AVL, Graz, Austria). Hemoglobin oxygen saturation was measured with a hemo-oximeter (Cooximeter, AVL). Hemoglobin concentration was assessed using the cyanmethemoglobin method.

Measurement of Sublingual Microcirculation

Sublingual microcirculatory flow index (MFI) for small ($<50\ \mu m$ according mainly capillaries) and medium vessels (between 50 and 150 μm according mainly to postcapillary venules) and capillary density were assessed with Sidestream Dark Field imaging (SDF; MicroScan, MicroVision Medical, Amsterdam, Netherlands) (Fig. 1). Sublingual microvascular blood flow was measured with a laser Doppler flowmeter (LDF; O2C, LEA Medizintechnik, Giessen, Germany), and capillary-venous hemoglobin oxygen saturation (HbO_2) was investigated with a tissue reflectance spectrophotometer (TRS; O2C).

SDF imaging is a noninvasive intravital microscope developed for assessment of the human microcirculation without using fluorescent dyes in clinical practice. The instrument consists of a small endoscopic-like light guide attached to a light source with filters. The examined tissue is illuminated with polarized light with a wavelength of 530 nm permitting optimal imaging of the microcirculation, because of identical light absorption of oxy- and deoxyhemoglobin at this wavelength. Within the tissue, light is scattered, depolarized, and reflected. Because the emitted light is primarily absorbed by hemoglobin, red cells can be remarkably well observed in all vascular. The illuminated light and reflected light travel via independent pathways. A five times magnifying lens is used to project the image onto a video camera. The movie is saved with an analog-digital converter in the computer for later off-line analysis.

The LEA device consists of a combination of a LDF and a tissue reflectance photometer. LDF is a noninvasive instrument permitting real-time measurement of microvascular perfusion, particularly in the skin. Monochromatic laser light with a wavelength of 820 nm penetrates the surface of a sampling volume and interacts with both static and moving cells (i.e., red blood cells). Because of the Doppler effect, photons of the laser light scattered on blood cells undergo a frequency shift proportional to the speed of the moving cells.

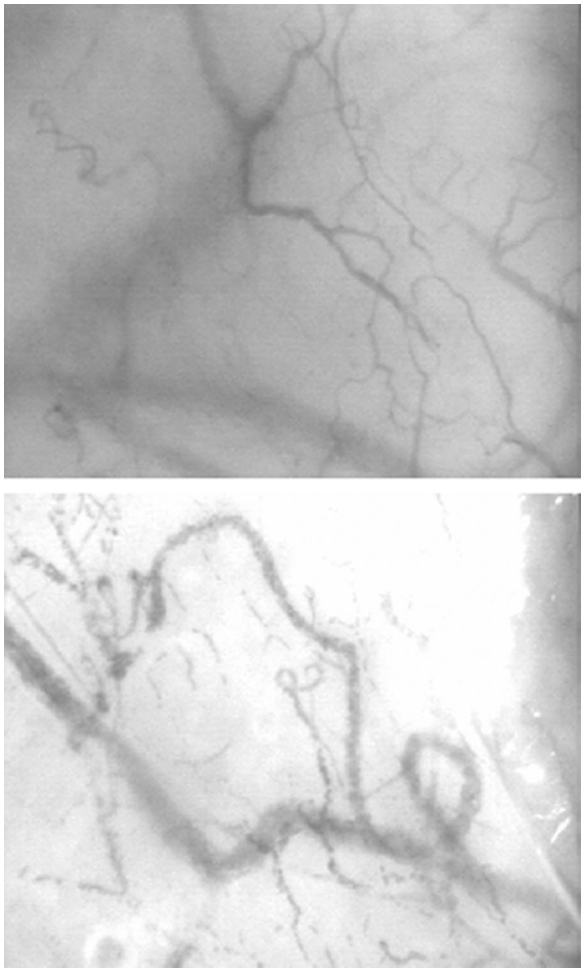

Fig. 1. *SDF imaging of the sublingual mucosal microvasculature of a pig after initiation of hemorrhagic shock (*upper*) and after fluid resuscitation (*below*).*

Backscattered light is transmitted via a flexible optical fiber to the laser Doppler photodiode, amplified, analyzed, and finally transformed into an analog signal. The magnitude and frequency distribution of changes in wavelength are proportional to the number of blood cells multiplied by the mean velocity of these cells.

TRS was originally introduced by Sato et al.[24] and advanced by Frank et al.[25] as a noninvasive method for assessing tissue hemoglobin oxygen saturation ($tHbO_2$) and changes in tissue hemoglobin concentration (tHbC). The light of a xenon high-pressure arc lamp is transferred to a tissue surface via a single highly flexible microlightguide with a diameter of 250 μm. Backscattered light is collected by six identical microlightguides (each 250 μm in diameter) arranged around the circumference of the illuminating lightguide. From there it travels to a rotating bandpass interference filter disk, which serves as a monochromating unit for a spectral range of 502 to 628 nm. This filter disk permits sampling in steps of 2 nm and a sampling rate of 100 spectra per second. The monochromatic light is transmitted to a photomultiplier tube, fed into a current-to-voltage converter and amplified by a cascade amplifier. The voltage signal is offset compensated, filtered by a low-pass filter, fed into an analog-to-digital converter, and transferred to a computer. The recorded spectra are balanced against a standard white reference produced by a mirror. Absolute values of $tHbO_2$ and relative values of hemoglobin concentration tHbC are calculated by using an algorithm, originally developed by Dümmler and described in detail by Frank et al.[25]

Experimental Protocol

Animals were randomized to a gelatin resuscitated group (gelatin, n = 10; Gelofusin, Braun Melsungen, Melsungen, Germany); a 6% HES 130/0.4 solution group (n = 10; Voluven, Fresenius Kabi, Graz, Austria); and a HS 7.2%-hydroxyethyl starch 6% (molecular weight, 200.000; hydroxyethylation ratio, 0.5) group (HS-HES; n = 10; Hyperhes, Fresenius Kabi, Graz, Austria).

Baseline measurements of systemic hemodynamics, blood gas variables, serum lactate levels, and microcirculatory parameters were performed after a resting period of about 1 hour after animal preparation (measurement point 1). Total blood volume in study animals was assumed as 70 mL/kg body weight. After baseline measurements, 60% of the calculated blood volume was withdrawn via the femoral venous catheter. Shed blood was directly transferred and processed in an automatic cellsaver system (CATS, Fresenius, Vienna, Austria) after anticoagulation with natriumcitrate 3.8% in a ratio of 1:9.

Fluid resuscitation was started after systemic and microcirculatory measurements had been repeated 30 minutes after initiation of hemorrhagic shock (measurement point 2). Animals were retransfused with either gelatin in a ratio of 1:1.3 to lost blood volume, HES with a ratio of 1:1 to shed blood volume, or 4 mL/kg body weight of HS-HES in accordance with the recommendations for fluid resuscitation in critical bleeding.[12,13] In addition, all animals received the washed cell saver concentrate for maintaining a critical red cell mass. After substitution, all values were measured again. The fluid volume transfused was recorded. After fluid resuscitation, systemic and regional measurements were repeated at 30 minutes after resuscitation without further interventions (measurement point 3).

Data Analysis and Statistics

Both devices were calibrated as recommended by the manufacturer before baseline measurement. A video sequence of 30 seconds was recorded with the SDF device and digitally recorded on a personal computer. The video files were evaluated off-line with a software program (MAS Analysis Software Version 2.1, MicroVision Medical, Amsterdam, Netherlands) in a blinded fashion. The MFI is a semiquantitative method to describe microvascular flow quality from the recorded movie. A picture of the video is divided in four quadrants with examples of vessel classifications:

Table 1 Systemic Hemodynamic Parameters

	BL Mean ± SD	Shock Mean ± SD	Resuscitation Mean ± SD	Time	Time-Group	Group
HR (beats/min)				$p < 0.001$	$p = 0.371$	$p = 0.306$
Gelatine	94 ± 28	185 ± 35*	132 ± 19*			
HES	85 ± 15	168 ± 47*	118 ± 25*			
HS-HES	95 ± 19	194 ± 36*	114 ± 22*			
MAP (mm Hg)				$p < 0.001$	$p = 0.258$	$p = 0.988$
Gelatine	69 ± 9	36 ± 8*	106 ± 13*			
HES	74 ± 10	32 ± 7*	104 ± 12*			
HS-HES	70 ± 19	40 ± 14*	101 ± 12*			
CVP (mm Hg)				$p < 0.001$	$p < 0.001$	$p = 0.500$
Gelatine	7 ± 4	4 ± 2*	13 ± 3*†			
HES	8 ± 2	4 ± 2*	14 ± 3*†			
HS-HES	9 ± 2	5 ± 1*	10 ± 2†			
CI ($mL \cdot kg^{-1} \cdot min^{-1}$)				$p < 0.001$	$p = 0.006$	$p = 0.326$
Gelatine	101 ± 27	51 ± 13*	231 ± 52*†			
HES	103 ± 22	57 ± 19*	205 ± 65*†			
HS-HES	109 ± 20	63 ± 15*	162 ± 41*†			
SVI ($mL \cdot kg^{-1} \cdot beat^{-1}$)				$p < 0.001$	$p = 0.492$	$p = 0.501$
Gelatine	1.1 ± 0.2	0.3 ± 0.1*	1.7 ± 0.2*			
HES	1.2 ± 0.2	0.4 ± 0.2*	1.8 ± 0.6*			
HS-HES	1.2 ± 0.3	0.3 ± 0.1*	1.5 ± 0.6			
MPAP (mm Hg)				$p < 0.001$	$p = 0.179$	$p = 0.397$
Gelatine	16 ± 4	12 ± 4	33 ± 10*			
HES	18 ± 4	16 ± 9	34 ± 9*			
HS-HES	19 ± 4	15 ± 5*	28 ± 7*			
PAOP (mm Hg)				$p < 0.001$	$p = 0.007$	$p = 0.979$
Gelatine	9 ± 2	8 ± 3	17 ± 3*†			
HES	11 ± 3	7 ± 3*	16 ± 6*†			
HS-HES	11 ± 3	9 ± 3	13 ± 3†			

Data are displayed as mean ± SD. Values were taken at baseline, after shock phase, and after fluid resuscitation in gelatine-, HES, or HS-HES resuscitated animals.

* Time effect $p < 0.05$.

† Group effect $p < 0.05$.

HR, heart rate; MAP, mean arterial blood pressure; CVP, central venous pressure; CI, cardiac index; SVI, stroke volume index; MPAP, mean pulmonary artery pressure; PAOP, pulmonary artery occlusion pressure.

small (10–25 μm) and medium (26–50 μm). Both, small and medium vessels in each quadrant are described as no flow with 0 scoring points, intermittent flow (1 point), sluggish flow (2 points), and continuous flow (3 points). The points of all four quadrants are summarized and divided by four to get the MFI.[26] The functional capillary density is described as the number of perfused capillaries in one image field.

LDF and TRS derived variables were recorded for a period of at least 100 seconds. Mean values of these variables were used for statistical comparison.

An analysis of variance for repeated measurements was performed to analyze differences in mean values between and within groups for systemic hemodynamics, systemic acid-base status, blood gas variables, serum lactate concentrations, and sublingual microcirculatory parameters. Global hypothesis was tested two-sided at the 0.05 significance level. In case of significant differences, further comparisons were made with paired *t*-tests within groups to baseline and between groups at individual time points. Data in text, tables, and figures are presented as mean ± SD, if not stated otherwise.

RESULTS

Systemic Variables During Hemorrhage

Hemorrhage caused significant changes in systemic hemodynamic parameters (Table 1). Mean arterial blood pressure, mean pulmonary arterial pressure, central venous pressure as well as pulmonary capillary wedge pressure and stroke volume index decreased significantly. Despite a compensatory significant increase in heart rate, cardiac index was reduced. Arterial lactate concentrations increased indicating some tissue hypoxia during hemorrhage in all study animals (Table 2).

Hemorrhage and Sublingual Microcirculatory Parameters

Hemorrhage resulted in a significant decrease in hemoglobin oxygen saturation (Fig. 2). Also functional capillary density decreased. The microvascular flow index in both, small and medium vessels, decreased because of a more flow or no flow or sluggish flow phenomenon during hemorrhagic shock.

Table 2 Arterial Blood Gas Variables, Hemoglobin Concentration, and Lactate Levels

	BL Mean ± SD	Shock Mean ± SD	Resuscitation Mean ± SD	Time	Time-Group	Group
pH				$p < 0.001$	$p = 0.674$	$p = 0.275$
Gelatine	7.51 ± 0.05	7.49 ± 0.05	7.41 ± 0.07*			
HES	7.50 ± 0.07	7.46 ± 0.07	7.38 ± 0.08*			
HS-HES	7.50 ± 0.10	7.44 ± 0.05	7.36 ± 0.06*			
pCO_2 (mm Hg)				$p < 0.001$	$p = 0.944$	$p = 0.236$
Gelatine	36 ± 4	34 ± 4	40 ± 6			
HES	37 ± 6	36 ± 6	40 ± 7			
HS-HES	39 ± 3	37 ± 4	43 ± 4*			
pO_2 (mm Hg)				$p = 0.338$	$p = 0.390$	$p = 0.258$
Gelatine	234 ± 20	228 ± 38	230 ± 27			
HES	217 ± 42	197 ± 30	217 ± 47			
HS-HES	220 ± 31	218 ± 33	207 ± 33			
BE				$p < 0.001$	$p = 0.510$	$p = 0.432$
Gelatine	4.8 ± 1.2	−2.6 ± 2.3*	−0.1 ± 3.2*			
HES	4.0 ± 3.1	−0.5 ± 2.7*	−1.3 ± 3.8*			
HS-HES	4.7 ± 2.1	−2.2 ± 1.5*	−0.8 ± 2.9*			
Hb (mg/dL)				$p < 0.001$	$p < 0.001$	$p < 0.001$
Gelatine	9.4 ± 0.8	8.3 ± 0.5*	6.3 ± 1.5*†			
HES	9.2 ± 0.7	8.2 ± 1.1*	6.7 ± 0.5*†			
HS-HES	9.3 ± 0.6	8.6 ± 0.5*	9.0 ± 0.5†			
Hct				$p < 0.001$	$p < 0.001$	$p = 0.001$
Gelatine	28 ± 3	25 ± 2*	19 ± 4*†			
HES	28 ± 4	24 ± 3*	20 ± 1*†			
HS-HES	28 ± 2	25 ± 1*	26 ± 1†			
Lactate (mmol/L)				$p < 0.001$	$p = 0.644$	$p = 0.786$
Gelatine	3.1 ± 1.5	5.2 ± 2.1*	3.8 ± 1.8			
HES	3.2 ± 1.1	5.4 ± 1.5*	4.5 ± 1.3			
HS-HES	2.8 ± 1.1	4.8 ± 1.0*	4.7 ± 1.7*			

Data are displayed as mean ± SD. Values were taken at baseline, after shock phase, and after fluid resuscitation in gelatine-, HES, or HS-HES resuscitated animals.

* Time effect $p < 0.05$.

† Group effect $p < 0.05$.

pCO_2, partial pressure of carbon dioxide; pO_2, partial pressure of oxygen; BE, baxe excess; Hb, hemoglobin; Hct, hematocrit.

Fluid Resuscitation and Systemic Variables

All systemic hemodynamic parameters returned or even exceeded to baseline values after calculated volume resuscitation in all three groups (Table 1). Despite "overcorrection" of mean arterial blood pressure, central venous pressure, and pulmonary capillary occlusion pressure, heart rate increased significantly when compared with baseline. Nevertheless, fluid resuscitation with HS-HES resulted in a significantly decrease in cardiac index because of a reduction in stroke volume index from reduced filling pressures when compared with gelatin or HES resuscitation. Hemodilution resulted in a decrease in hematocrit and hemoglobin concentrations (Table 2). In animals receiving HS-HES, serum lactate levels stayed significantly increased after fluid resuscitation.

Fluid Resuscitation and Sublingual Microcirculatory Parameters

Fluid resuscitation led to a restoration of all microcirculatory parameters determined in gelatin and HES resuscitated animals and almost all parameters except microvascular hemoglobin oxygen saturation in animals treated with HS-HES (Fig. 2). Microvascular blood flow and microvascular hemoglobin oxygen saturation even exceeded above baseline values in animals treated with HES and gelatin, but not in animals resuscitated with HS-HES. This phenomenon was accompanied with a significant increase in microvascular flow index in small vessels as a result of capillary recruitment.

DISCUSSION

Animals resuscitated with 4 mL/kg HS-HES experienced an attenuated microcirculatory blood flow and a decreased tissue oxygen supply in the sublingual area when compared with gelatin or HES resuscitated hemorrhagic pigs. This finding on the microcirculatory level mirrors the effects of the different fluids administered on systemic hemodynamic parameters, where isotonic colloids showed superior hemodynamic effects compared with HS-HES in standardized volume regimes.

Hemorrhage and Systemic and Microcirculatory Hemodynamics

Hemorrhagic shock has detrimental effects on systemic hemodynamics, as described previously.[5,27] Blood loss of

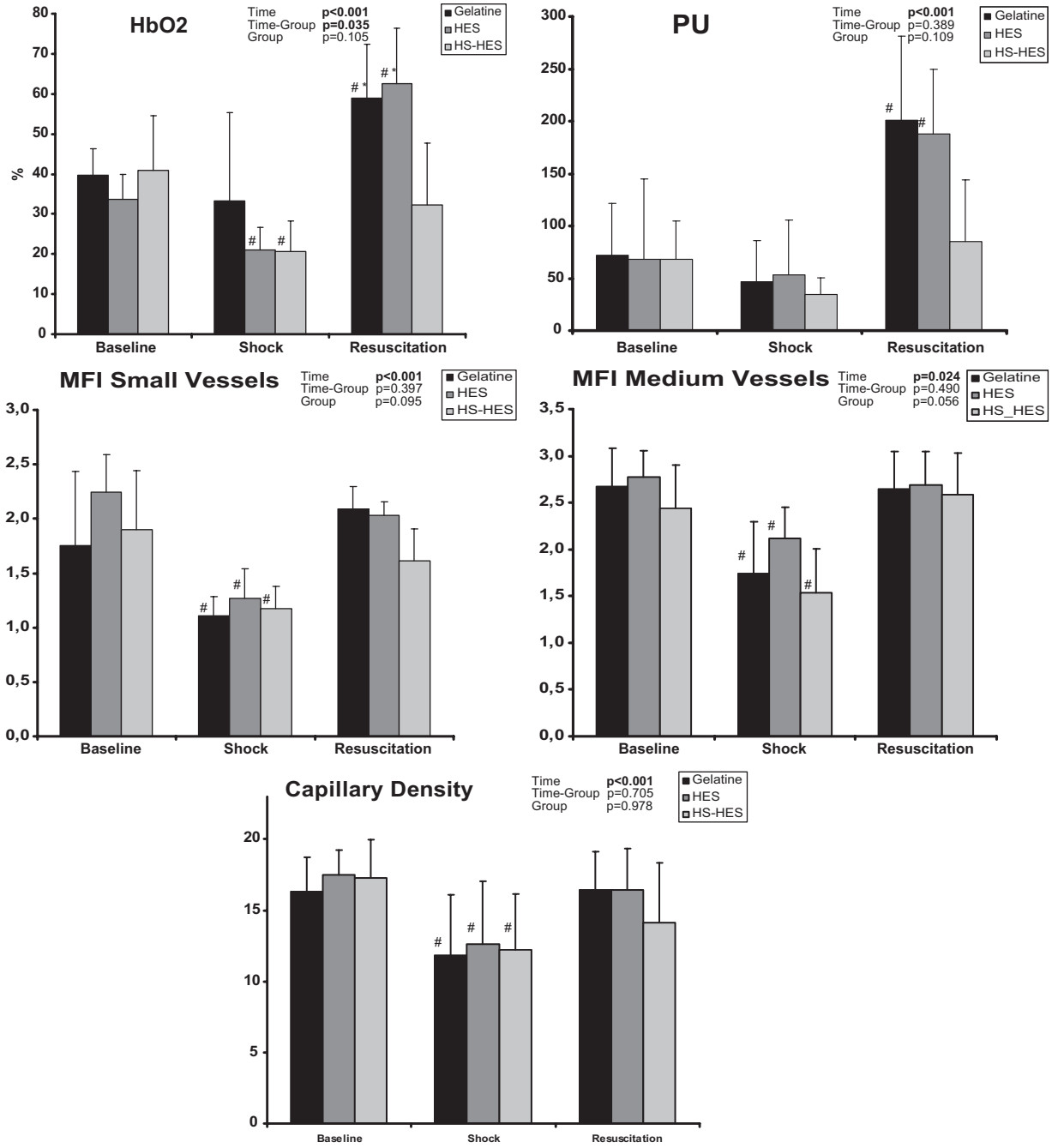

Fig. 2. *Sublingual mucosal microcirculatory parameters assessed with SDF imaging, laser Doppler flowmetry, and tissue reflectance photometry. Data are displayed as mean ± SD. Values were taken at baseline, after shock phase, and after fluid resuscitation in gelatin-, HES, or HS-HES resuscitated animals. HbO_2, microvascular hemoglobin oxygen saturation; PU, perfusion units; MFI, microvascular flow index; #time effect* $p < 0.05$*; *group effect* $p < 0.05$.

more than half of the calculated blood volume decreased both, perfusion pressure and systemic blood flow. The consequence of the macrohemodynamic deterioration was a significant reduction in microcirculatory blood flow. A reduced flow decreased the microvascular flow index in vessels with a diameter of less than 50 μm because of stagnation in blood flow and a stop and go phenomenon in the capillaries. As tissue oxygen supply is a function of both, arterial oxygen content and blood flow, oxygen delivery to the sublingual area decreased. As a consequence of this reduction in oxygen supply, a higher proportion of deoxygenated hemoglobin in the sublingual microcirculation was detected, indicating an increased oxygen extraction ratio of the tissue. This assumption can be made, as the tissue spectrophotometer detects microcirculatory hemoglobin saturation, mainly in the postcapillary venules, where about 85% of the microcirculatory

blood volume is present.[28,29] The deterioration of the microcirculation led to some tissue hypoxia, indicated by an increase in systemic serum lactate levels. Our data are in line with results by Dubin et al.[30] in which hemorrhage-induced hyperlactatemia and a decrease in systemic oxygen delivery evidenced anerobic metabolism.

Fluid Resuscitation and Systemic Hemodynamics

Systemic hemodynamics returned above baseline values after fluid resuscitation in all three groups. The dosages administered were considered to be equipotent in their volume effect. However, administration of the two isotonic colloidal fluids resulted in higher filling pressures concomitant with better systemic blood flow when compared with HS-HES. Gelatin solution shows a volume efficacy of about 70%, whereas that of HES 130/0.4 is reported to be 100%.[13] Thus, in the present study pigs received a median dose of 50 mL/kg gelatin solution, 41 mL/kg HES, or 4 mL/kg HS-HES. Furthermore, it is known that each milliliter administered, HS-HES mobilizes an additional 7 mL of free water from the interstitial and intracellular space into the intravascular compartment, resulting in a net volume amount of 32 mL/kg.[31] This may be the reason for the different systemic hemodynamic end points presented in the present study. The recommendation to use 4 mL/kg HS or colloidal fluid for fluid resuscitation is based on several animal studies. Wade et al.[32] have previously shown that 4 mL/kg of HS or dextran in severe hemorrhage in swine produced a survival rate of 66%. The same study group investigated the optimal dose of HS or dextran and observed a difference in survival time between hemorrhaged pigs resuscitated either with 4 or with 11.5 mL/kg, which failed to be statistically significant.[33] They concluded that the dose of 4 mL/kg alone may not be fully effective for ensuring survival but does extend survival time after severe hemorrhage.

Looking on the data presented, the survival rate in all three groups is 100%, and systemic hemodynamic parameters returned to or above baseline values after fluid administration. The only conclusion that can be made is that fluid administration in the acute phase after hemorrhage should be targeted on systemic hemodynamic variables and not on general fluid volume administration recommendations.

Fluid Resuscitation and Sublingual Microcirculation

In the present experiment, effects of resuscitation on systemic hemodynamics are mirrored in the determined microcirculatory parameters. Restoration of perfusion pressure and systemic blood flow led to an increase in microcirculatory blood flow, which was markedly pronounced in isotonic colloidal fluids. This nearly threefold increase in microcirculatory blood supply in conjunction with a better flow quality in the small- and medium-sized microvessels resulted in a clear increase in microvascular hemoglobin saturation in gelatin and HES animals, whereas microvascular hemoglobin saturation in HS-HES-treated animals even decreased after resuscitation. One explanation for this observation is the marked difference in systemic hemodynamics. The higher systemic blood flow because of isotonic colloids supplies the microcirculation better with oxygenated blood. Nonetheless, the low microcirculatory hemoglobin oxygen saturation might also mirror an inadequate oxygen supply/demand ratio, despite restoration in microcirculatory blood flow and flow index. As described above, venous blood clearly dominates the catchment volume of the tissue spectrophotometer, so that the measured mixed value always positively measures the oxygen saturation value from the venous end of the capillaries.[28,29] As we can assume that the oxygen supply was restored after resuscitation, low microcirculatory hemoglobin oxygen saturation indicates an increased tissue oxygen extraction ratio, when compared with the two isotonic colloidal fluids.

Results regarding improvement of regional blood flow and oxygen supply because of administration of hypertonic or hyperoncotic fluids are mainly based on studies comparing crystalloid fluids.[34,35] These results do cease to exist when hypertonic or hyperoncotic fluids were compared with colloids. Reinhart et al.[36] investigated oxygen uptake in dogs after resuscitation with either HS-HES or 6% HES alone. HES demonstrated a significant better systemic oxygen uptake when compared with the HS-HES solution after blood pressure restoration. A further study examined oxygen delivery and consumption of hypertonic or hyperoncotic fluid compared with crystalloids, dextran and HS in hemorrhagic pigs.[37] In this study, no differences in systemic oxygen transport variables were detected. An investigation by Braz et al.[38] showed significantly lower intramucosal pH values in an hemorrhagic shock dog model resuscitated either with HS or HS/6% dextran when compared with lactated Ringer's solution or HES resuscitation. These investigations are, partially, in line with the results of the present study. Focusing on the sublingual microcirculation, which shares a similar embryologic origin with the digestive mucosa, colloidal fluid administration led to a better restoration of microvascular hemoglobin oxygen saturation than a hypertonic or hyperoncotic fluid resuscitation.

Clinical Relevance and Limitations of the Study

Our data suggest that both, isotonic and hypertonic colloidal infusions may be useful in resuscitation of hemorrhagic shock in restoration systemic hemodynamics, microcirculatory blood flow and flow quality. But only isotonic colloids are able to improve hemoglobin oxygen saturation at the microcirculatory level. However, these results can only be extrapolated to humans and especially to patients with hemorrhagic shock with great caution. When using a specific fluid solution for resuscitation one has to consider possible side effects like anaphylactic reactions, increased bleeding tendency, alterations in the immune function, or renal dysfunction, which have all been reported for colloid solutions.[6,7]

Furthermore, regional measurements in one organ may not be representative for other organs or even for different tissue compartments in the same organ.[39] However, the sublingual mucosal microcirculation shares the embryologic origin with the digestive mucosa, a mucosa highly vulnerable to systemic hemodynamic and microcirculatory deterioration. A recent investigation of Creteur et al.[40] could demonstrate a significant correlation between sublingual mucosal carbon dioxide pressure, microvascular flow index, and gastric mucosal carbon dioxide pressure. For that reason, sublingual assessment of the microcirculation is a reliable and easy to performable access path not only in the experimental setting, but also at the bed side.

The baseline fluid requirement during general anesthesia was calculated with 4 $mL \cdot kg^{-1} \cdot h^{-1}$ i.v. throughout the whole experiment. Taking into account that only ¼ of the administered crystalloid fluid remains in the circulation, it is possible that the administration of the baseline fluid contributes to a more pronounced edema formation in the interstitium with a consequent deterioration of oxygen supply to the tissue.[5] Considering that animals resuscitated with HS-HES had lower filling pressures when compared with the isotonic colloidal fluid groups, this could be a further possible explanation for the decrease of microvascular hemoglobin oxygen saturation in the present investigation.

The main limitation of the presented study is the fact that the resuscitation end points were not cardiac filling pressures but fixed volume regimens thought to be equipotent in volume restoration. Administration of gelatin and isotonic HES resulted in significantly higher filling pressures when compared with hypertonic HES. This increase in cardiac preload led to an improvement in systemic blood flow. We have to assume that this increase in cardiac output is mirrored in the improved microvascular blood flow in animals treated with either gelatin or HES compared with HS-HES animals. The better restored microcirculatory blood flow in the sublingual area may induce a more pronounced tissue oxygen extraction ratio indicated by the significantly reduced microvascular HbO_2 in animals receiving HS-HES. Furthermore, the different volume applications for resuscitation led to differences in hematocrit. Animals resuscitated with HS-HES have shown an increased hemoglobin concentration and hematocrit level compared with gelatin or isotonic HES animals. Low values of hematocrit suggest that oxygen transport to tissue may be compromised by diminished oxygen-carrying capacity.[41] This fact was not observed in the present study. In contrary, animals resuscitated with an isotonic colloidal fluid observed diminished hematocrit levels, but experienced higher venular HbO_2 levels indicating a sufficient oxygen delivery to the tissue. And animals resuscitated with HS-HES experienced higher hematocrit levels and an increased tissue oxygen extraction ratio. A possible explanation is the fact of better microcirculatory blood rheology because of reduced blood viscosity.[42] The apparent viscosity of blood in the microvasculature depends on the existing shear forces and is determined among plasma viscosity and red blood cell aggregation by hematocrit.[42] In the present study, resuscitation with either gelatin or isotonic HES resulted in superior intravascular volume resuscitation with the consequence of probably better viscosity because of reduced shear stress. The enhanced rheological characteristics may also contribute to the improved microvascular blood flow pattern and, therefore, to oxygen supply to the tissue compared with animals receiving HS-HES. These limitations of the present study of different end points used and different amounts of fluids infused make it difficult to interpret the present data and to make a clear conclusion. Therefore, we cannot exclude, if other dosages of fluids targeting on macrohemodynamic parameters lead to similar microcirculatory effects.

Again, a major limitation of the present study is the fact that resuscitation targets of animals were not filling pressures, but standardized fluid volume regimens in dosages considered primarily to be equipotent. Despite initially creating this study design based on general recommendations for fluid volume administration to mimic the fluid management in patients with hemorrhagic shock after bleeding control, hemodynamic end points differed between groups. Animals resuscitated with either gelatin or isotonic HES had a resuscitation advantage on a macroscopic level, resulting in an "unfair fight" between groups. This difference in turn may lead to the difference seen at the microvascular level, as mentioned above. In other words, inadequate overall systemic fluid resuscitation after hemorrhage led to inadequate microvascular HbO_2 and probably not some inherent property of HS-HES. We cannot exclude, if other dosages of fluids targeting on macrohemodynamic parameters lead to similar microcirculatory effects. Therefore, fluid administration should be targeted on systemic hemodynamic variables and not on general fluid volume administration recommendations.

CONCLUSION

On the basis of the data presented here, it appears that severely hemorrhaged swine can be hemodynamically stabilized with either isotonic or hypertonic colloidal fluids. The volume of the fluid administration in the acute phase of hemorrhagic shock after bleeding control should be targeted on systemic hemodynamic variables and not on general fluid volume administration recommendations. The main finding of the present investigation is an adequate restoration of sublingual microcirculatory blood flow and flow quality within all three study groups, but only gelatin and HES improved microvascular hemoglobin oxygen saturation. This observation might be a consequence of an inadequate oxygen supply/demand ratio, despite restoration in microcirculatory blood flow and flow index after HS-HES resuscitation, but has its limitation because of superior hemodynamic effects of isotonic colloids compared with HS-HES in standardized volume regimes.

REFERENCES

1. Bisonni RS, Holtgrave DR, Lawler F, Marley DS. Colloids versus crystalloids in fluid resuscitation: an analysis of randomized controlled trials. *J Fam Pract.* 1991;32:387–390.
2. Choi PT, Yip G, Quinonez LG, Cook DJ. Crystalloids vs. colloids in fluid resuscitation: a systematic review. *Crit Care Med.* 1999; 27:200–210.
3. Velanovich V. Crystalloid versus colloid fluid resuscitation: a meta-analysis of mortality. *Surgery.* 1989;105:65–71.
4. Schierhout G, Roberts I. Fluid resuscitation with colloid or crystalloid solutions in critically ill patients: a systematic review of randomised trials. *BMJ.* 1998;316:961–964.
5. Knotzer H, Pajk W, Maier S, et al. Comparison of lactated Ringer's, gelatine and blood resuscitation on intestinal oxygen supply and mucosal tissue oxygen tension in haemorrhagic shock. *Br J Anaesth.* 2006;97:509–516.
6. Nolan J. Fluid replacement. *Br Med Bull.* 1999;55:821–843.
7. Boldt J. Fluid choice for resuscitation of the trauma patient: a review of the physiological, pharmacological, and clinical evidence. *Can J Anaesth.* 2004;51:500–513.
8. Wade CE, Grady JJ, Kramer GC. Efficacy of hypertonic saline dextran fluid resuscitation for patients with hypotension from penetrating trauma. *J Trauma.* 2003;54:S144–S148.
9. Kreimeier U, Bruckner UB, Niemczyk S, Messmer K. Hyperosmotic saline dextran for resuscitation from traumatic-hemorrhagic hypotension: effect on regional blood flow. *Circ Shock.* 1990;32: 83–99.
10. Kreimeier U, Brueckner UB, Schmidt J, Messmer K. Instantaneous restoration of regional organ blood flow after severe hemorrhage: effect of small-volume resuscitation with hypertonic-hyperoncotic solutions. *J Surg Res.* 1990;49:493–503.
11. Maningas PA. Resuscitation with 7.5% NaCl in 6% dextran-70 during hemorrhagic shock in swine: effects on organ blood flow. *Crit Care Med.* 1987;15:1121–1126.
12. Thompson R, Greaves I. Hypertonic saline-hydroxyethyl starch in trauma resuscitation. *J R Army Med Corps.* 2006;152:6–12.
13. Boldt J. Volume replacement in the surgical patient—does the type of solution make a difference? *Br J Anaesth.* 2000;84:783–793.
14. Spahn DR, Cerny V, Coats TJ, et al. Management of bleeding following major trauma: a European guideline. *Crit Care.* 2007; 11:412.
15. Lehr HA, Bittinger F, Kirkpatrick CJ. Microcirculatory dysfunction in sepsis: a pathogenetic basis for therapy? *J Pathol.* 2000;190:373–386.
16. Dhainaut JF, Shorr AF, Macias WL, et al. Dynamic evolution of coagulopathy in the first day of severe sepsis: relationship with mortality and organ failure. *Crit Care Med.* 2005;33:341–348.
17. Deitch EA, Dayal SD. Intensive care unit management of the trauma patient. *Crit Care Med.* 2006;34:2294–2301.
18. Rackow EC, Falk JL, Fein IA, et al. Fluid resuscitation in circulatory shock: a comparison of the cardiorespiratory effects of albumin, hetastarch, and saline solutions in patients with hypovolemic and septic shock. *Crit Care Med.* 1983;11:839–850.
19. Rittoo D, Gosling P, Bonnici C, et al. Splanchnic oxygenation in patients undergoing abdominal aortic aneurysm repair and volume expansion with eloHAES. *Cardiovasc Surg.* 2002;10:128–133.
20. Lang K, Boldt J, Suttner S, Haisch G. Colloids versus crystalloids and tissue oxygen tension in patients undergoing major abdominal surgery. *Anesth Analg.* 2001;93:405–409, 403rd contents page.
21. Vollmar MD, Preissler G, Menger MD. Small-volume resuscitation restores hemorrhage-induced microcirculatory disorders in rat pancreas. *Crit Care Med.* 1996;24:445–450.
22. Vajda K, Szabo 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;36:338–344.
23. Messmer K, Kreimeier U. Microcirculatory therapy in shock. Resuscitation 1989;18(suppl):S51–S61.
24. Sato N, Kamada T, Shichiri M, Kawano S, Abe H, Hagihara B. Measurement of hemoperfusion and oxygen sufficiency in gastric mucosa in vivo. Evidence of mucosal hypoxia as the cause of hemorrhagic shock-induced gastric mucosal lesion in rats. *Gastroenterology.* 1979;76:814–819.
25. Frank KH, Kessler M, Appelbaum K, Dümmler W. The Erlangen micro-lightguide spectrophotometer EMPHO I. *Phys Med Biol.* 1989; 34:1883–1900.
26. Boerma EC, Mathura KR, van der Voort PH, Spronk PE, Ince C. Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. *Crit Care.* 2005;9:R601–R606.
27. Lomas-Niera JL, Perl M, Chung CS, Ayala A. Shock and hemorrhage: an overview of animal models. *Shock.* 2005;24(suppl 1):33–39.
28. Burton A. The vascular bed. In: *Physiology and Biophysics of the Circulation*. Chicago, IL: Year Book Medical; 1965.
29. Gandjbakhche AH, Bonner RF, Arai AE, Balaban RS. Visible-light photon migration through myocardium in vivo. *Am J Physiol.* 1999; 277:H698–H704.
30. Dubin A, Estenssoro E, Murias G, et al. Effects of hemorrhage on gastrointestinal oxygenation. *Intensive Care Med.* 2001;27:1931–1936.
31. Reed RL II, Johnston TD, Chen Y, Fischer RP. Hypertonic saline alters plasma clotting times and platelet aggregation. *J Trauma.* 1991;31:8–14.
32. Wade CE, Hannon JP, Bossone CA, et al. Resuscitation of conscious pigs following hemorrhage: comparative efficacy of small-volume resuscitation. *Circ Shock.* 1989;29:193–204.
33. Wade CE, Dubick MA, Grady JJ. Optimal dose of hypertonic saline/dextran in hemorrhaged swine. *J Trauma.* 2003;55:413–416.
34. Reed LL, Manglano R, Martin M, Hochman M, Kocka F, Barrett J. The effect of hypertonic saline resuscitation on bacterial translocation after hemorrhagic shock in rats. *Surgery.* 1991;110:685–688; discussion 688–690.
35. Behrman SW, Fabian TC, Kudsk KA, Proctor KG. Microcirculatory flow changes after initial resuscitation of hemorrhagic shock with 7.5% hypertonic saline/6% dextran 70. *J Trauma.* 1991;31:589–598; discussion 599–600.
36. Reinhart K, Rudolph T, Bredle DL, Cain SM. O_2 uptake in bled dogs after resuscitation with hypertonic saline or hydroxyethylstarch. *Am J Physiol.* 1989;257:H238–H243.
37. Chiara O, Pelosi P, Brazzi L, et al. Resuscitation from hemorrhagic shock: experimental model comparing normal saline, dextran, and hypertonic saline solutions. *Crit Care Med.* 2003;31:1915–1922.
38. Braz JR, do Nascimento P Jr, Paiva Filho O, et al. The early systemic and gastrointestinal oxygenation effects of hemorrhagic shock resuscitation with hypertonic saline and hypertonic saline 6% dextran-70: a comparative study in dogs. *Anesth Analg.* 2004; 99:536–546, table of contents.
39. Rowell L. *General Principles of Vascular Control.* New York: Oxford University Press; 1986.
40. Creteur J, De Backer D, Sakr Y, Koch M, Vincent JL. Sublingual capnometry tracks microcirculatory changes in septic patients. *Intensive Care Med.* 2006;32:516–523.
41. Lipowsky HH. Microvascular rheology and hemodynamics. *Microcirculation.* 2005;12:5–15.
42. Baskurt OK, Meiselman HJ. Blood rheology and hemodynamics. *Semin Thromb Hemost.* 2003;29:435–450.