Small-volume hypertonic saline/pentastarch improves ileal mucosal microcirculation in experimental peritonitis

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Abstract

We compared the effects of hypertonic saline 7.2%/6% hydroxyethyl starch (HSS-HES) and isotonic saline 0.9%/6% hydroxyethyl starch (ISS-HES) on ileal microcirculatory blood flow (MBF) at the initial phase of septic shock. Pigs were anesthetized and mechanically ventilated. Catheters were inserted into right atrium, pulmonary artery, carotid artery, and portal vein for hemodynamic measurements and for blood sampling. Ileal mucosal and muscularis MBF was continuously measured by laser Doppler flowmetry (LDF). Septic shock was obtained 240 min after induction of fecal peritonitis; then animals were randomized to receive 10 mL.kg^{-1} during 10 min of either HSS-HES or ISS-HES. Systemic and microcirculatory blood flow as well as systemic metabolism were assessed. Fecal peritonitis promoted a hypodynamic septic shock, with significant reduction of mean arterial pressure (MAP) and cardiac index (CI). Ileal mucosal MBF (-34%) and ileal muscularis MBF (-54%) significantly diminished from baseline. Contrary to ISS-HES group, mucosal MBF significantly augmented after HSS-HES (+192% at min 150 post-shock) despite low blood pressure. There was weak correlation with CI (r^2= 0.2, P=0.01) . Muscularis MBF didn't change. HSS-HES-treated animals had a significantly higher osmolarity and sodium concentration than ISS-HES group. Other variables did not change. Small-volume resuscitation with HSS-HES, but not ISS-HES, improved ileal microcirculatory impairment in experimental peritonitis model of septic shock even when MAP was low. This beneficial microcirculatory effect could be valuable in the management of early severe sepsis.

Introduction

During conventional management of severe sepsis, both isotonic crystalloid and colloid have been used to restore systemic hemodynamic status, but the volume of isotonic fluid required to restore cardiocirculatory function is large and often promoting partial and transient benefits, which are especially poor at the splanchnic bed.[1] Since 1980, when hyperosmotic 7.5% sodium chloride (NaCl) was first described as a small-volume resuscitation solution,[2] many clinical and animal studies were published. Hemodynamic effects of hypertonic saline resuscitation in hemorrhagic shock are well documented. Hypertonic saline solution (HSS) promotes both systemic and microcirculatory blood flow improvement,[3] reduces bacterial translocation and lung injury after hemorrhage,[4,5] diminishes neutrophil rolling and adherence to endothelium and reduces in vivo vascular leakage,[6] enhances intracellular killing of bacteria while attenuating receptor-mediated activation of proinflammatory cascades.[7,8] Although experience with the use of hypertonic saline solutions in sepsis is limited; these interesting benefits were started to be applied in sepsis.[9]

In addition, although vascular volume expansion occurs rapidly after infusion of HSS, volume expansion is relatively transient unless an oncotically effective colloid (dextran or hydroxyethyl starch) is added in order to preserve the intravascular volume gain.[10] Contrary to dextran, no study has evaluated the effects of hypertonic saline/hydroxyethyl starch (HSS-HES) and isotonic saline/ hydroxyethyl starch (ISS-HES) in initial treatment of septic shock. Thus, our aim was to compare HSS-HES with ISS-HES on systemic, regional and microcirculatory blood flow as well as systemic metabolism in experimental peritonitis model of septic shock.

Materials and Methods

Animal preparation

The animal ethic committee of the University of Claude-Bernard-Lyon 1, Lyon, France, approved the protocol. Fourteen domestic female pigs (weight, 25 kg) were fasted overnight but were allowed free access to water. Pigs were sedated with intramuscular injection of ketamine (10mg.kg^{-1}). Anesthesia was induced with propofol (3 mg.kg^{-1}), and was maintained with sevoflurane (MAC=2), sufentanyl (1 μg.kg^{-1}.hr^{-1}) and cisatracurium at doses of 0.3 mg.kg^{-1} every 30 min. After performing a tracheostomy, animals were mechanically ventilated with a FIO_2 that maintained an arterial partial pressure of O_2 (PaO_2) above 80 torr, at a tidal volume of 8-10 mL.kg^{-1}, and respiratory rate that maintained $PaCO_2$ at 40±5 torr. End-expiratory pressure was zero.

Catheters were inserted into right carotid artery, for continuous monitoring of MAP and for collecting blood samples, and into right atrium through left external jugular vein for fluid and drug administration. A 7-Fr thermister-tip catheter (Baxter Edwards Critical-Care, Ivrine, CA) was advanced into pulmonary artery through right external jugular vein to measure mean pulmonary artery pressure (MPAP, mmHg), CVP (mmHg), PCWP (mmHg) and cardiac index (CI, mL.min^{-1}.kg^{-1}), and to draw mixed venous blood samples. Location of pulmonary arterial catheter tip was determined by observing the characteristic pressure trace on the monitor as it was advanced through right heart into pulmonary artery. The central venous temperature was monitored with a thermistor in the pulmonary artery. Temperature of the animal was maintained at 37.2 (±) 0.8°C with a heating pad and heat lamps. During surgical preparation, animals were given a continuous infusion of 15-20 mL.kg^{-1}.h^{-1} lactated Ringer solution to maintain CVP and PCWP values at 6-8 mmHg.

A midline laparotomy was performed. The spleen was removed to avoid autotransfusion during shock[11] and a catheter was inserted into portal vein for blood sampling.[12] 0.75 g.kg^{-1} of autologous feces was collected through a small incision of antimesenteric border of the ascending colon, to be used later to induce fecal peritonitis and septic shock. Bowel incision was then closed with continuous sutures. A 5 cm segment of distal ileum, 20 cm proxi-

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mal to ileocecal junction, was defined. A platform was placed above the abdomen and fixed to a ring stand on either side of the pig. This platform has mobile and adjustable two-pieces. The isolated ileal segment was opened along its entire antimesenteric border and exteriorized on ventral midline of the animal. The cut edges of ileum were stitched to the two-pieces of a platform. The height of the platform above the ventral abdominal wall was adjusted, and the two-pieces were positioned in such a way that mucosa of opened bowel was well exposed, but the mesentery supplying the segment was not compressed. A Laser Doppler flowmetry (LDF) probe was suspended, and positioned perpendicularly on intestinal mucosa with help of special miniholder (Perimed®, Probe 407 with PH 07 Miniholder); by this way, LDF probe would generate minimal pressure on tissue surface and artefacts caused by respiratory movements and intestinal peristalsis would be eliminated (extract of LDF recording Figure 1). In the same way, another LDF probe was placed on ileal serosa with help of a special miniholder (Perimed®, Probe 407 with PH 07 Miniholder). Exteriorized ileal segment was covered with humidified cotton and gauze, and temperature of gut was maintained with warm water and a heat lamp. After surgical preparation was completed, two large-bore tubes with multiple side-holes were placed in abdominal cavity for later induction of peritonitis, and the laparotomy incision was approached.

Hemodynamics and oxygen utilization

All intravascular pressure measurements were referenced to midchest level. Cardiac output was measured by thermo-dilution method. Three consecutive determinations of CO were made by injecting 10 mL of ice-cold bolus of 5% dextrose at end expiration. The mean value recorded as CO. Heart rate, MAP, MPAP, PCWP, CVP and core temperature were continuously measured. Systemic pulse pressure variation (PPV) was calculated as the difference of maximal and minimal pulse pressure divided by their mean on one respiratory cycle.[13] Arterial, mixed venous, and portal vein blood samples were drawn for determinations of blood gases (ABL 520, Radiometer, Copenhagen, Denmark), and for lactate concentration. Hemoglobin concentration (g/dL) was determined by standard spectrophotometric method. NaCl concentration was determined by indirect potentiometry (mmol.L^{-1}) and osmolarity (mosmol.L^{-1}) was determined by freezing point depression.

Ileal mucosal and muscularis blood flow

MBF was continuously measured with LDF,[14] a well-characterized technique for measurement of red blood cell flux in the tissues[11,15] and because the optical probe has a spatial resolution of 0.5^{-1} mm3, the obtained measurements reflect only the flow on surface where the probe is applied and not the entire intestinal wall.[16] LDF signals were exported via analog output and acquired on line via a multichannel interface (Mac Paq MP100; Biopac system Inc., Goleta, CA) with acquisition/analysis software (Acqknowledge 7.2; Biopac system Inc.) to a portable computer. Two different signals are available for external recording. One output is proportional to intensity of backscattered light. This signal is useful to determine whether optical probe is making adequate contact with tissue surface. The second signal is proportional to blood flow, which has been shown to scale linearly with independent measurements of local perfusion in a variety of tissues. LDF are not calibrated to measure absolute blood flow, but indicate MBF in arbitrary perfusion units (PU). Before each experiment, a calibration using random Brownian motion of small scatters in an emulsion (Periflux motility standard, Perimed) was performed. The quality of LDF signal was controlled online by visualizing it on a computer screen, so that motion artefacts and noise due to inadequate probe attachment could be immediately detected and corrected before measurements started. Each reading was the mean of two min of LDF tracing.

Experimental design

Animals were stabilized for 60 min before baseline measurements (TB) were performed (Figure 2). Sepsis was induced by injecting 0.75 g.kg^{-1} of autologous feces suspended in 200 mL of warm isotonic saline through the abdominal tubes in the peritoneal cavity. After induction of peritonitis, animals received 10 mL.kg^{-1}.h^{-1} of Ringer lactate. Regional and local blood flow was continuously monitored. Systemic hemodynamic variables were meas-

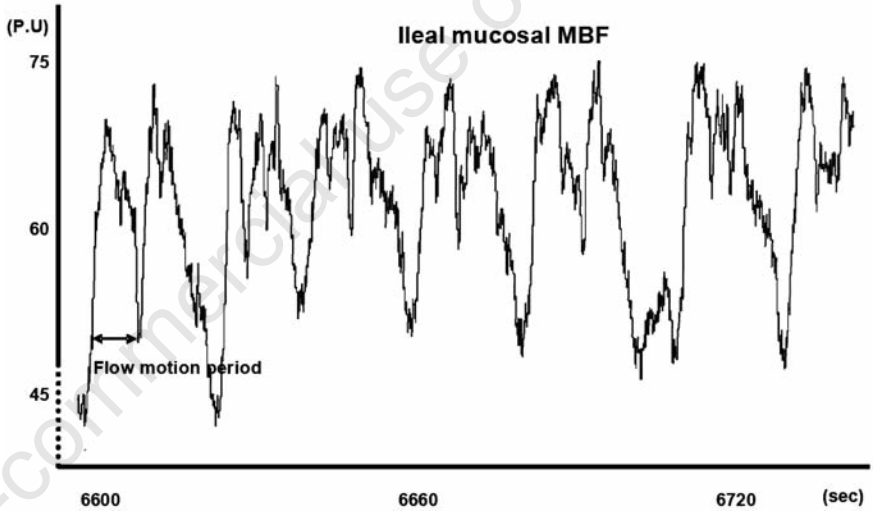

Figure 1. Extracts from 120 seconds of ileal microcirculatory blood flow (MBF) tracing, by LDF in arbitrary perfusion units (PU). Note the absence of respiratory oscillations and peristaltic artefacts. Flow motion oscillations are present with frequency of 4 to 6 per min.

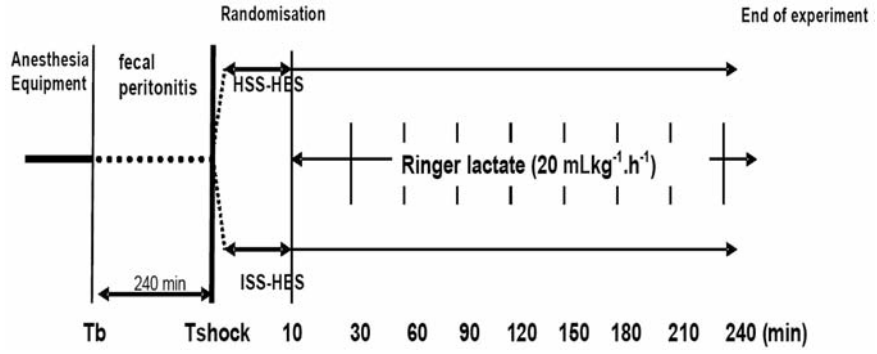

Figure 2. Experimental protocol. After the equipment of pigs, septic shock was induced by fecal peritonitis. Then animals were randomised into two groups to receive equal volumes of either hypertonic saline 7.2%/hydroxyethyl starch (HSS-HES) or isotonic saline 0.9%/hydroxyethyl starch (ISS-HES); Animals were observed for 240 min.

ured hourly. Resuscitation was started at T shock, when MAP or CI was reduced by 50% from its baseline (TB) value. They were randomly assigned into two groups just before intervention, thereby minimizing any potential risk of biased handling of animals; the studied group (HSS-HES, n=7) received hypertonic saline 7.2%/6% hydroxyethyl starch, 200/0.5 (HyperHES®, Fresenius Kabi, France) and the control group (ISS-HES, n=7) received isotonic saline 0.9%/6% hydroxyethylstarch, 130/0.4 (VOLUVEN®, Fresenius Kabi, France) in raison of 10 mL.kg^{-1} infused within 10 min, through a central venous line.

In addition all animals received constant intravenous infusion of 20 mL.kg^{-1}.h^{-1} of Ringer lactate. Microcirculatory blood flow was continuously monitored. Systemic hemodynamic variables were taken at the moment of septic shock (T shock), after 10 min (T10 min) and half-hourly (T30, T60, T90, T120, T150, T180, T210 and T240 min), while blood samples were taken hourly (at T60, T120, T180 and T240 min) during four hours after start of resuscitation.

After completing the experiment, all animals were sacrified with an intravenous injection of potassium chloride (20 mmol) under deep anesthesia.

Statistical analysis

Because of the large variability of baseline values, measurements of LDF are usually expressed as changes relative to baseline. Data were analyzed using a two-way analysis of variance for repeated measurements followed by a Newman-Keuls procedure. A P value <0.05 was considered statistically significant. A multiple linear regression was used to evaluate variations of MBF compared to variations of CI during HSS-HEA infusion. All values are expressed as mean±SD, except when mentioned otherwise. We used Sigma Stat 3.1 software for Windows.

Results

All animals completed the 10-hours study protocol.

The animal peritonitis model

After 240 min of its induction, fecal peritonitis promoted hemodynamic changes that are characteristic of a hypodynamic septic shock. There were significant (P <0.05) reductions of MAP, CI, CVP, PCWP and augmentation of PPV. There were severe and significant (P <0.05) stepwise impairment of ileal mucosal MBF (-34%) and ileal muscularis MBF (-54%) from baseline (Figure 3). Hemodynamic, ileal microcirculatory blood flow variables during peritonitis are presented in Table 1.

Hemodynamic variables during fluid administration

During HSS-HES and ISS-HES fluid administration, CI increased significantly up to min 90, and then it was progressively decreased. CI under HSS-HES infusion was higher (P <0.05) up to min 30 (+37%). MAP significantly increased up to min 90 during HSS-HES infusion and up to min 30 during ISS-HES infusion with significant difference between the two groups at 30 min and then MAP progressively deteriorated in both groups. CVP was increased during HSS-HES infusion (P <0.05) without significant difference between the two groups. There were no significant differences in PPV and PCWP between the two groups. Hemodynamic variables during fluid infusion are presented in Table 2.

Microcirculatory variables during fluid administration

Ileal mucosal MBF (Figure 4) was significantly augmented after HSS-HES up to min 150 (+119%) compared to shock value, when it was progressively diminished to reach its TB value after 240 min. Meanwhile, Ileal mucosal MBF after ISS-HES insignificantly augmented and approached its T shock value after 240 min. Mucosal MBF was higher under HSS-HES infusion until the end of the experiment with significant difference between the two groups up to min 120 post shock (226% *versus* 88% of shock value). Ileal muscularis MBF were insignificantly increased in both groups during this time and without difference between both groups. The correlation between variation of mucosal MBF and CI was poor (r^2= 0.2,

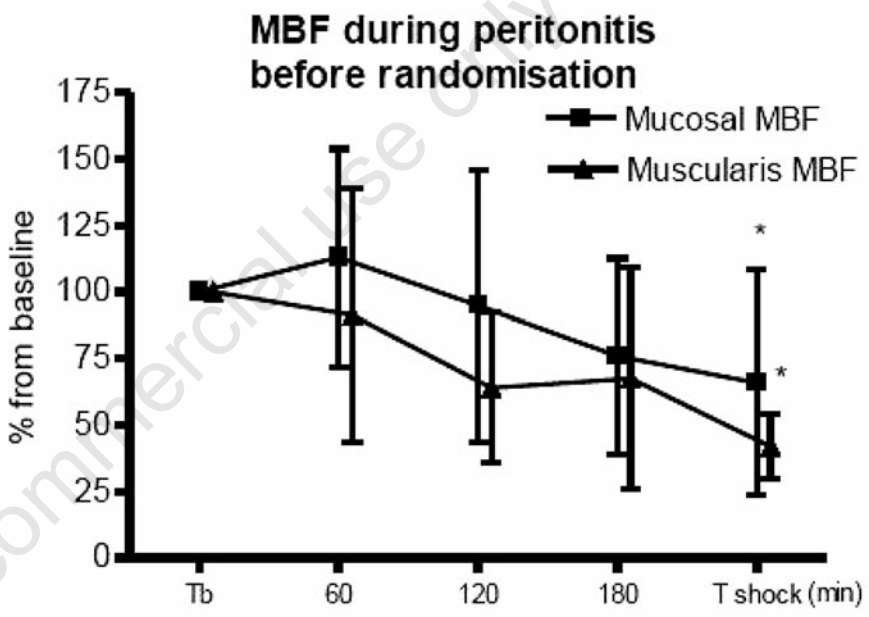

Figure 3. Ileal mucosal and muscularis microcirculatory blood flow (MBF) changes during peritonitis. Peritonitis was induced at TB (baseline). MBF were set at 100% at TB. Values at 60, 120, 180 and 240 (T240=onset of septic shock) min were expressed as percentage of their baseline values. Data are presented as mean±SD. * P <0.05 (different from baseline).

Table 1. Hemodynamic data during peritonitis. TB, baseline value before induction of peritonitis. Values were taken at 60, 120, 180 and 240 (onset of septic shock) min after induction of peritonitis; MAP, mean arterial pressure; CI, cardiac index; HR, heart rate; CVP, central venous pressure; PCWP, pulmonary capillary wedge pressure; VPP, pulse pressure variation; MPAP, mean pulmonary artery pressure.

Time (min)	Baseline	T60	T120	T180	T240
MAP (mmHg)	83±9	64±10*	57±8*	50±12*	48±9*
CI (ml.kg^{-1}min^{-1})	73±17	54±15*	45±12*	39±12*	32±14*
HR (beats/min)	95±13	109±24*	128±33*	144±40*	160±39*
CVP (mmHg)	7±2	6±3*	6±3*	5±3*	5±3*
PCWP (mmHg)	9±2	6±2*	6±2*	6±2*	5±2*
PPV (%)	11±5	20±7*	29±20*	30±10*	33±9*
MPAP (mmHg)	21±4	20±5	20±4	19±3	19±3

Data are presented as mean±SD. *P <0.05 (significant difference from baseline)

Table 2. Hemodynamic variables during fluid administration. After induction of peritonitis, septic shock corresponded to T shock, when pigs were resuscitated either with (HSS-HES) hypertonic saline 7.2%/hydroxyethyl starch or (ISS-HES) isotonic saline 0.9%/hydroxyethyl starch.

Time (min)		Tshock	T10	T30	T60	T90	T120	T150	T180	T210	T240
MAP.	ISS	44±6	61±7*	54±6*	52±6	52±7	49±10	49±10	47±12	46±12	46±13
(mmHg)	HSS	40±9	67±13*	66±10*#	56±6*	55±11*	48±5	45±8	45±7	43±8	36±9
CI	ISS	31±5	51±15*	53±14*	49±13*	47±19*	38±13	36±18	33±15	30±12	32±19
($ml.kg^{-1}min^{-1}$)	HSS	30±8	89±25*#	73±17*#	62±24*	55±24*	42±15	40±14	34±16	34±18	34±22
CVP	ISS	7±3	8±4	8±4	8±3	7±3	6±3	8±4	6±3	6±3	6±2
(mmHg)	HSS	4±2	9±3*	9±4*	8±3*	7±3	5±2	6±3*	6±3	7±3*	8±3*
PCWP	ISS	6±3	10±3*	10±5*	9±3*	8±3	7±4	7±3	8±2	7±2	8±2
(mmHg)	HSS	5±2	10±2*	9±3*	7±2	7±2	7±2	6±2	7±3	7±2	7±2
PPV	ISS	29±12	14±4*	18±6*	17±7	20±11	26±13	25±8	21±5	19±2	22±6
(%)	HSS	35±9	11±5*	15±5*	22±8*	29±13	27±10	26±8	26±9	28±10	26±11
MPAP	ISS	20±3	28±3*#	23±2	23±4#	23±4	24±2	24±5	25±5	25±5*	27±4*
(mmHg)	HSS	17±4	22±4	20±4	18±3	19±4	20±6	21±6	22±7	24±8*	24±7*

MAP, mean arterial pressure; CI, cardiac index; CVP, central venous pressure; PCWP, pulmonary capillary wedge pressure; MPAP, mean pulmonary artery pressure; PPV, pulse pressure variation. Data are presented as mean±SD. *P <0.05 (significant difference from T shock). #P <0.05 (significant difference between groups).

P=0.01). The ratio between mucosal MBF and CI during resuscitation was not different from both groups. Microcirculatory blood flow variables during fluid infusion are presented in Table 2.

Acid-base status during fluid infusion

Arterial and portal vein lactates did not change significantly over the course of time in ISS-HES and HSS-HES groups and there was no significant difference between the two groups. Arterial pH and bicarbonates as well as portal vein bicarbonates were within the normal limits at the beginning of resuscitation and stayed as such till the end of the experiment in both groups. Arterial and portal vein pH did not show significant differences between the two groups. Results are presented in Table 3.

Table 3. Acid-base balance. After induction of peritonitis, septic shock took place at T shock, when pigs were resuscitated during 240 min either with (HSS-HES) hypertonic saline 7.2%/hydroxyethyl starch or (ISS-HES) isotonic saline 0.9%/hydroxyethyl starch.

Time (min)		T shock	T60	T120	T180	T240
Arterial lactate	ISS	1.96±0.57	2.26±0.89	2.06±0.31	2.04±0.52	2.62±1.86
(mmol/L)	HSS	2.95±2.21	2.90±2.5	2.43±1.20	2.45±1.52	3.60±2.91
Portal vein lactate	ISS	2.80±1.30	2.86±1.16	2.94±1.20	2.93±1.22	4.54±2.64
(mmol/L)	HSS	3.92±2.32	2.41±0.63	2.46±0.95	2.09±0.89	3.33±1.51
Arterial bicarbonates	ISS	38±3	39±4	41±6	37±6	38±6
(mmol/L)	HSS	41±6	40±9	39±7	41±5	42±5
Portal vein bicarbonates	ISS	30±2	27±3	26±4	28±2	26±4
(mmol/L)	HSS	27±2	26±2	27±3	26±3	24±4
Arterial pH	ISS	7.44±0.03	7.44±0.03	7.42±0.04	7.43±0.05	7.41±0.03
	HSS	7.38±0.05	7.36±0.07	7.39±0.06	7 38±0.04	7.35±0.08
Portal venous pH	ISS	7.35±0.04	7.39±0.03	7.37±0.03	7.37±0.04	7.32±0.07
	HSS	7.27±0.03	7.33±0.04	7.26±0.03	7.28±0.01	7.30±0.05
Sodium	ISS	135±4	135±2	135±2	134±2	134±3
($mmol.L^{-1}$)	HSS	135±3	149±4*#	148±4*#	146±3*#	145±2*#
Osmolarity	ISS	284±6	285±5	285±7	283±6	283±9
($mosmol.L^{-1}$)	HSS	284±6	311±7*#	292±37	303±10*#	270±83

Data are presented as mean±SD. *P <0.05 (significant difference from T shock). #P <0.05 (significant difference between groups).

Sodium concentration and osmolarity during fluid infusion

Plasma osmolarity after 180 min of resuscitation was moderately higher in the HSS-HES group than in the ISS-HES group (283±9 *vs.* 303±10, P <0.05) as well as plasma sodium at the end of the experiment (145±2 *vs.* 134±3, P <0.05). Results are presented in Table 3.

Variables of oxygenation during fluid administration

Oxygen delivery was significantly increased after 60 min in both groups compared to shock values without significant difference between the two groups. Oxygen consumption did not significantly change in both groups. These modifications were accompanied with augmentation of oxygen extraction (P <0.05) in both groups after 60 and 120 min of septic shock and without significant difference between the two groups. Mixed venous oxygen saturation ($SmvO_2$) was augmented (P <0.05) in both groups after 120 min without difference between the groups. Portal vein oxygen saturation ($SpvO_2$) was augmented significantly during ISS-HES infusion compared to shock value. Hemoglobin concentration was stable in both groups and there was no difference between groups. Hemoglobin and variables of oxygenation are shown in Table 4.

Discussion

Our main finding in this study is that small-volume resuscitation with HSS-HES improved ileal mucosal microcirculatory impairment after septic shock caused by experimental peritonitis to find its baseline (TB) values at the end of the experiment (Figure 5). On the contrary, resuscitation with equal volume of ISS-HES, despite an initial non-significant improvement, mucosal MBF decreased progressively below shock values. In fact, HSS-HES-treated animals have approximately doubled the Doppler signal compared to ISS-HES.

Interestingly, during HSS-HES infusion, improvement of mucosal MBF was maintained till the end of the experiment although MAP and CI were only preserved up to min 90. These beneficial effects on macrocirculation have not been confirmed on non septic animals.[17] In our study, CVP was also better preserved with HSS-HES than with ISS-HES, probably due to mobilization of fluids from intracellular to extracel-

lular compartment by the osmotic gradient produced by HSS-HES. Moreover, the correlation between MBF and systemic hemodynamic was very weak (r^2=0.2 for CI). For the rest, Figure 5 shows a tendency of a better amelioration of MBF than CI during resuscitation with HSS-HES than with ISS-HES. We can then postulate that HSS-HES have a specific microcirculatory effect. Similarly, Zakaria *et al.*[18] demonstrated this effect by using an in vivo videomicroscopy and optical Doppler velocimetry on intestinal villi in a hemorrhagic shock model. They found that hypertonic saline resuscitation improves intestinal perfusion by selective vasodilatation of precapillary arterioles even at MAP close to shock levels. In accordance to our study, this vasodilatation may also occur during HSS-HES infusion. This demonstrates the interest of using hypertonic saline resuscitation during the early phase of severe sepsis and septic shock to combat tissue ischemia even if MAP is still low.

Our data confirm a study showing that under a combination of hypertonic saline (7.5%) with a dextran, MBF was augmented during the first two hours of resuscitation compared to an association of isotonic saline plus a dextran.[15] However, authors used a model of (endotoxemia) septic shock which is more averted from human septic shock than ours (experimental peritonitis). Moreover, they used another colloid and did not study the correlation between variations of systemic output and mucosal MBF. Somell *et al.* showed an improvement of survival in a swine endotoxemia model under a combination of hypertonic saline (7.5%) with a dextran, but authors did not study any microcirculatory effect.[19] In their study, they used isotonic saline as control, therefore making it difficult to separate the beneficial effect of either HSS or colloid.

During peritonitis (before resuscitation), there were different reductions of microcirculatory blood flow in the ileal mucosa (-34%) and ileal muscularis (-59%). It seems possible that blood flow was deviated from muscularis towards the mucosa during peritonitis which had reached its maximum at the onset of shock. On the other hand, after resuscitation with HSS-HES, there was a higher augmentation of MBF in the mucosa than in the muscularis. These blood flow modifications can be explained by the redistribution of blood flow between ileal mucosa and muscularis during shock and after resuscitation for the same. This muscularis ischemia might be responsible for paralytic ileus observed in septic shock. Contrary to our study, Revelly *et al.*[20] had observed an augmentation of mucosal MBF during endotoxic shock possibly because of intensive fluid resuscitation.

Among the ISS-HES group, portal vein oxygen saturation (SO_2) was significantly augmented. This might be due to the phenomenon

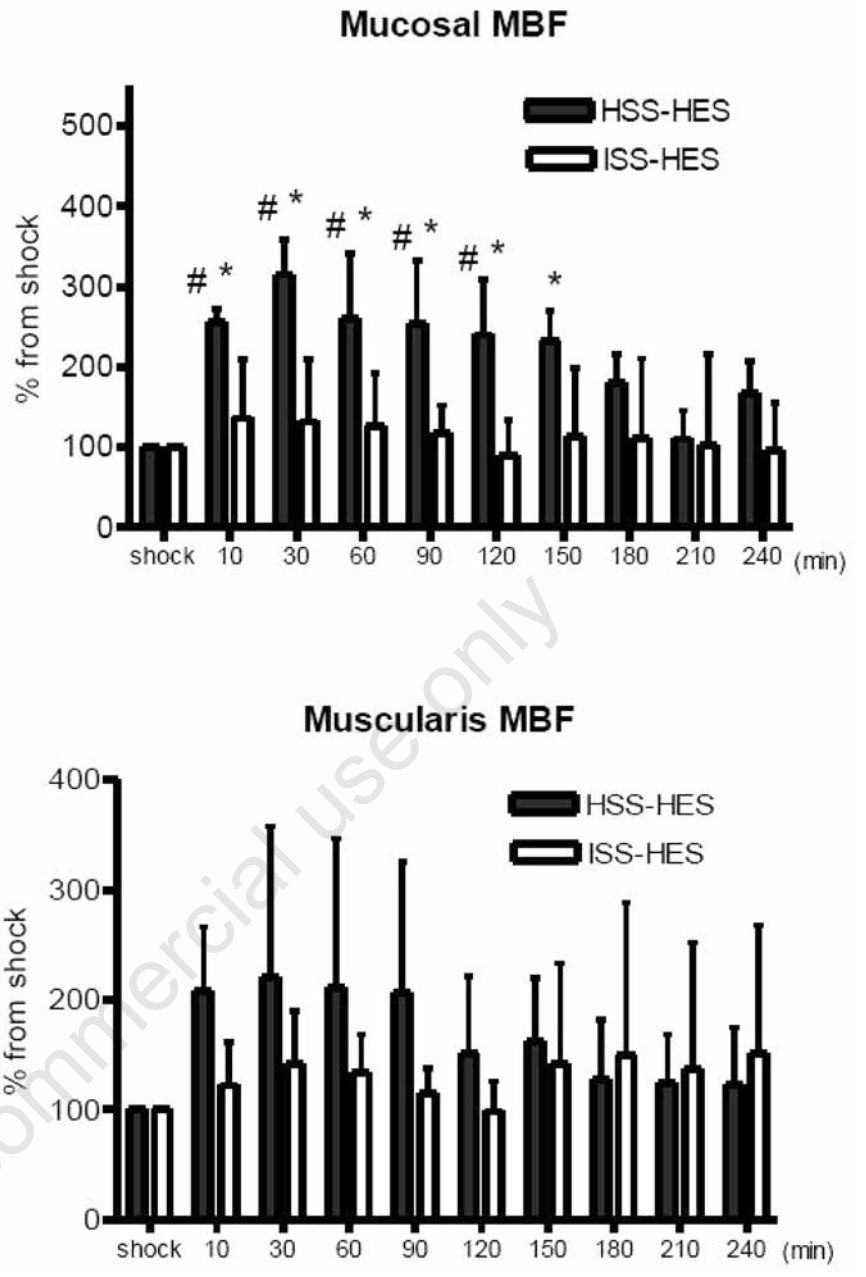

Figure 4. Relative ileal mucosal and muscularis microcirculatory blood flow changes during fluid administration after the onset of septic shock. Pigs were resuscitated with either hypertonic saline 7.2%/ hydroxyethyl starch (HSS-HES) or isotonic saline 0.9%/hydroxyethyl starch (ISS-HES). All microcirculatory blood flows (MBF) were set at 100% before fluid administration at T shock and measured values were expressed as percentage of T shock. Data are presented as mean±SD. *P <0.05 significant difference from T shock; #P <0.05 significant difference between groups.

of capillary shunting,[21] in which blood had shunted from precapillary arterioles to postcapillary venules without passing through the tissue capillaries. This shunting might have been caused by lack of proper precapillary dilatation during ISS-HES infusion contrary to HSS-HES infusion when portal vein SO_2 was not augmented. For the same reason, while portal vein lactate was slightly augmented during ISS-HES infusion, portal vein lactate was slightly improved till T180 min during HSS-HES infusion (Table 3). All these modifications during HSS-HES infusion might be explained by the augmentation of splanchnic MBF, regional blood flow redistribution toward mucosal tissue and/or due to an effect at cellular level that enables tissues to increase their oxygen extraction as oxygen supply is lowered.

During the current study, plasma osmolarity and sodium levels were increased significantly with a single infusion of hypertonic saline solution and this effect lasted during the whole

duration of the experiment. The potential risk of inducing important hypertonic states with possible harmful consequences to patients must be kept in mind and looked into, particularly after repetitive infusions of hypertonic saline solutions.[22]

One advantage of this study is the reproducibility of its results. In this regard, during the whole duration of this experiment, we were able to measure ileal MBF by LDF continuously at one precised point by fixing the exposed ileal mucosa on a platform (see materials and methods); so that any artefact caused by respiratory and peristaltic movements was eliminated. Hemoglobin was stable during the whole duration of the experiment, therefore avoiding any false readings resulting from modification of red blood cell count, as the LDF probe detects movement of red blood cells in relation to fixed tissues. Likewise, animals were resuscitated in the same manner, thereby avoiding any bias resulting from fluid loading difference between the two groups.

An important limit of this study is the exteriorisation of the ileum. Despite the precautions that were taken to keep the exposed intestine moist and warm, this approach may be less physiological. Nevertheless, these LDF tracings were reproducible and reliable. Furthermore, the LDF tracings were quite physiological with rhythmical oscillations (vasomotion) and these became more obvious after onset of sepsis (Extract of LDF recording Figure 1). Nevertheless, LDF measures a small volume of MBF (one mm^3 approximately) which includes arterioles, capillaries and veinules. Therefore we were not able to distinguish potential variations of microcirculation heterogeneity between both groups.

Another apparent limit is that animals were not fully resuscitated, despite oxygen extraction ratio (O_2ER) was augmented 60 min after resuscitation in both groups. However, our aim was to show if there might be a significant difference or not between the two groups (ISS-HES and HSS-HES) by utilizing equal volumes of fluid. Furthermore as this experiment was done to study a physiologic effect; accordingly, hemodynamic results were the consequences rather than the end points of resuscitation. In this way, we can easily judge if a significant difference is due to the type of fluid rather than to its quantity.

Other possible limits of our study are the small number of animals and the short observation time. Consequently, it was impossible to analyze the impact of fluid resuscitation on the development of multiple organ dysfunction and survival. Generally speaking, endotoxemia models present often a shorter survival;[19] therefore affording a worse survival analysis.

Although the potential beneficial effect of small volume resuscitation with hypertonic solutions has been extensively studied in hypovolemic shock, but animal and clinical studies that have been directed at treatment of septic shock using hypertonic solutions are limited. A chronic model to evaluate the potential beneficial effects of hypertonic saline solutions during longer period, in addition to its utilization during the early stages of septic shock has not been investigated. Recently in their review, Oliveira *et al.* commented on the mechanisms of action of hypertonic saline and discussed the use of hypertonic solutions for treatment of septic shock and they pointed out the potential benefits of using hypertonic solutions in patients with sepsis.[9] These improvements following hypertonic saline resuscitation might have a greater impact on the subsequent course of patients admitted to the intensive care unit. Indeed, Rivers *et al.*[23] showed that early goal-directed fluid resuscitation in patients with septic shock provides significant benefits related to outcome in the first 6 hours after admission to the emergency department. Our findings can be exploited in this sense with the utilization of HSS-HES during early resuscitation to augment microcirculatory blood flow and to improve tissue oxygenation.

Table 4. Variables of oxygenation during fluid administration. After induction of peritonitis, septic shock took place at T shock, when pigs were resuscitated during 240 min either with (HSS-HES) hypertonic saline 7.2%/ hydroxyethyl starch or (ISS-HES) isotonic saline 0.9%/hydroxyethyl starch. DO_2=oxygen delivery, VO_2=mesenteric oxygen consumption, ER=oxygen extraction, SO_2=oxygen saturation, CI=cardiac index.

Time (min)		T shock	T60	T120	T180	T240
Mixed venous-SO_2 (%)	ISS	54±12	67±8*	66±11*	60±10	56±16
	HSS	55±13	76±4*	68±8	64±8	56±17
Portal vein-SO_2 (%)	ISS	60±7	79±6*	77±11*	68±18	77±11
	HSS	71±9	83±9	68±5	67±8	71±16
DO_2 ($mL.min^{-1}.m^{-2}$)	ISS	4.77±1.07	6.56±1.82*	5.33±1.33	4.63±2.33	4.58±2.33
	HSS	4.16±1.40	8.40±0.10*	7.26±1.58	5.62±0.98	4.13±3.51
VO_2 ($mL.min^{-1}.m^{-2}$)	ISS	2.54±0.18	2.20±0.96	1.91±0.83	1.89±1.00	1.87±0.66
	HSS	1.60±0.41	1.55±0.22	1.91±0.29	1.96±0.43	1.36±0.25
O_2ER (%)	ISS	0.51±0.22	0.32±0.09*	0.33±0.11*	0.40±0.10	0.43±0.16
	HSS	0.43±0.12	0.22±0.03*	0.29±0.08*	0.35±0.09	0.43±0.20
CI /O_2ER	ISS	2±1	4±2*	4±2*	2±1	2±2
	HSS	2±1	7±3*	4±2*	2±1	2±2
Hemoglobin (g/dL)	ISS	11±1	9.5±1.6*	9.6±1.6*	9.5±2*	9.9±1.9*
	HSS	11±1.6	9.1±0.8*	9.6±0.7*	9.6±1.5*	9.6±1.5*

Data are presented as mean±SD. *P <0.05 (significant difference from T shock). #P <0.05 (significant difference between groups).

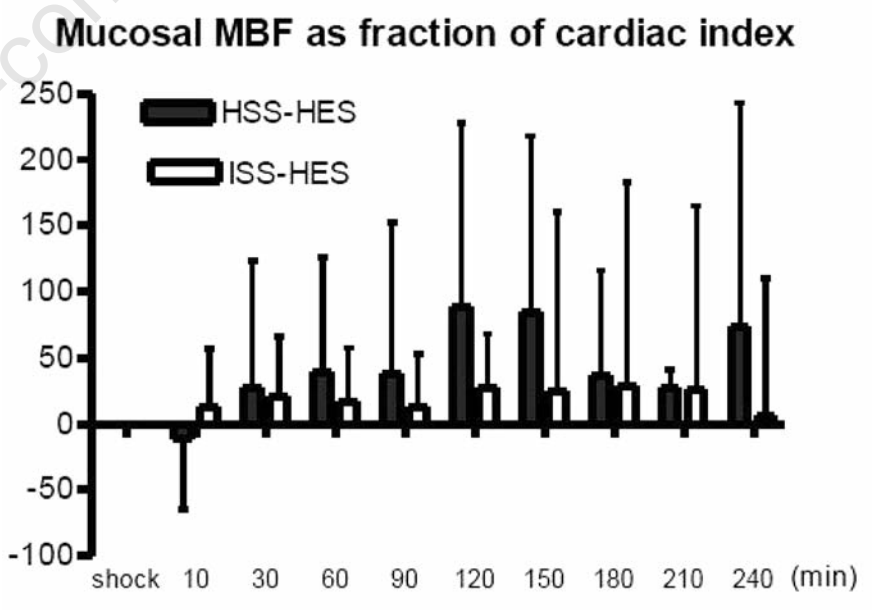

Figure 5. Changes in mucosal microcirculatory blood flows (MBF) compared with changes in cardiac index during fluid administration after the onset of septic shock. Pigs were resuscitated with hypertonic saline 7.2%/hydroxyethyl starch (HSS-HES) or isotonic saline 0.9%/hydroxyethyl starch (ISS-HES). The ratio between mucosal MBF and cardiac index at shock was set at 0. Data are presented as mean±SD

Conclusions

Resuscitation with HSS-HES during early phase of severe sepsis, compared with ISS-HES, augmented ileal mucosal microcirculatory blood flow even when MAP was low. Muscularis MBF was not augmented, maybe by redistribution of flow towards mucosa. The potential long-term beneficial effects of early infusions of HSS-HES during the course of chronic model of severe sepsis need further studies, including the potential impact on the mortality and the possible risk of induced hypertonic states.

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