Goal-directed Colloid Administration Improves the Microcirculation of Healthy and Perianastomotic Colon

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Background: The aim of this study was to compare the effects of goal-directed colloid fluid therapy with goal-directed crystalloid and restricted crystalloid fluid therapy on healthy and perianastomotic colon tissue in a pig model of colon anastomosis surgery.

Methods: Pigs (n = 27, 9 per group) were anesthetized and mechanically ventilated. A hand-sewn colon anastomosis was performed. The animals were subsequently randomized to one of the following treatments: R-RL group, 3 ml · kg⁻¹ · h⁻¹ Ringer lactate (RL); GD-RL group, 3 ml · kg⁻¹ · h⁻¹ RL + bolus 250 ml of RL; GD-C group, 3 ml · kg⁻¹ · h⁻¹ RL + bolus 250 ml of hydroxyethyl starch (HES 6%, 130/0.4). A fluid bolus was administered when mixed venous oxygen saturation dropped below 60%. Intestinal tissue oxygen tension and microcirculatory blood flow were measured continuously.

Results: After 4 h of treatment, tissue oxygen tension in healthy colon increased to 150 ± 31% in group GD-C versus 123 ± 40% in group GD-RL versus 94 ± 23% in group R-RL (percent of postoperative baseline values, mean ± SD; P < 0.01). Similarly perianastomotic tissue oxygen tension increased to 245 ± 93% in the GD-C group versus 147 ± 58% in the GD-RL group and 116 ± 22% in the R-RL group (P < 0.01). Microcirculatory flow was higher in group GD-C in healthy colon.

Conclusions: Goal-directed colloid fluid therapy significantly increased microcirculatory blood flow and tissue oxygen tension in healthy and injured colon compared to goal-directed or restricted crystalloid fluid therapy.

dynamic parameters, on intestinal and pulmonary wet/dry ratio, and on regional metabolism as measured by microdialysis.

Materials and Methods

After approval from the Animal Ethics Committee of the City of Bern, Switzerland, and in accordance with the Swiss National Institutes of Health guidelines for the care and use of experimental animals, we studied 27 healthy Swiss landrace pigs.

The pigs were premedicated with xylazine (2 mg · kg\(^{-1}\)) and intramuscular ketamine (20 mg · kg\(^{-1}\)). An ear vein was subsequently cannulated with an intravenous catheter for the administration of medications and fluid. For induction of anesthesia, midazolam 0.4 mg · kg\(^{-1}\) and 1 mg of atropine were administered. After induction, the pigs were intubated orally and ventilated with oxygen in air (FIO\(_2\) = 0.3). For maintenance of anesthesia, midazolam 0.5 mg · kg\(^{-1}\) · h\(^{-1}\), fentanyl 15 μg · kg\(^{-1}\) · h\(^{-1}\), pancuronium 0.3 mg · kg\(^{-1}\) · h\(^{-1}\), and propofol 0.15 mg · kg\(^{-1}\) · h\(^{-1}\) were administered continuously. The pigs were ventilated with a volume-controlled ventilator (Servo 900C; Siemens, Munich, Germany). Tidal volume was kept at 8–10 ml · kg\(^{-1}\), the respiratory rate was adjusted (20–24 breaths · min\(^{-1}\)) to maintain end-tidal carbon dioxide tension (Paco\(_2\)) at 40 ± 4 mmHg, and the positive end-expiratory pressure was 5 mmHg. After induction of anesthesia, all animals received 1.5 g of IV cefuroxim as an antibiotic prophylaxis. Animal stomachs were emptied with a large-bore orogastric tube. Body temperature of the animals was maintained at 38.0 ± 0.5°C with a warming mattress and a patient air warming system (Warm Touch 5700; Mallinckrodt, Neustadt, Germany).

Surgical Preparation

For direct arterial blood pressure monitoring, an arterial catheter was inserted in the carotid artery. A balloon-tipped pulmonary artery catheter was inserted via the right external jugular vein.

For further invasive monitoring and anastomosis surgery, a midline laparotomy was performed. A bladder catheter was inserted via a small incision in the bladder wall.

For assessment of microcirculatory blood flow, Laser Doppler flow probes (LDF; Oxford Optronix, Oxford, United Kingdom) were sutured through small incisions to the mucosa of healthy and perianastomotic colon as previously described. Two additional Laser Doppler flow probes were sutured to healthy and perianastomotic colon muscularis. Each LDF probe was secured with six microsutures to ensure close contact with the region of interest and to prevent motion disturbance due to respiration and peristaltic movements. All colonic incisions were subsequently closed with continuous sutures.

Polarographic tissue oxygen tension probes were inserted into healthy and perianastomotic colon tissue between the serosal and the mucosal tissue planes. The perianastomotic microdialysis catheter was inserted 2 cm proximal of the anastomosis. Finally, the perianastomotic vessels were ligated until the perianastomotic tissue oxygen tension measured 20–30 mmHg to reach “critical” tissue oxygen tension values. The abdominal incision was closed, and the pigs were allowed to stabilize for 30 min. A baseline measurement was performed during this time. After 30 min, all hemodynamic measurements were repeated every 30 min for 4 h. Blood samples were drawn after stabilization and hourly during treatment, after the measurements of hemodynamic parameters.

During catheter insertion and anastomosis surgery, all animals received 3 ml · kg\(^{-1}\) · h\(^{-1}\) of Ringer lactate (RL), reflecting a typical, restricted fluid replacement therapy. The pigs were then assigned to one of three fluid treatment groups using a reproducible set of computer-generated random numbers. The assignments were kept in sealed, opaque, and sequentially numbered envelopes until used. Treatment was initiated 15 min after the baseline measurement was performed.

Fluid Treatment Groups

The fluid treatment groups were established as follows: Restricted RL (Group R-RL), fixed rate of 3 ml · kg\(^{-1}\) · h\(^{-1}\) RL; goal-directed crystalloid therapy (Group GD-RL), fixed rate of 3 ml · kg\(^{-1}\) · h\(^{-1}\) RL, if mixed venous oxygen saturation was <60%, a 250-ml bolus of RL was administered with a 30-min lockout time between two boluses; goal-directed colloidal therapy (Group GD-C), fixed rate of 3 ml · kg\(^{-1}\) · h\(^{-1}\) RL, if mixed venous oxygen saturation was <60%, a 250-ml bolus of colloid (hydroxyethyl-starch 130/0.4) was administered (30-min lockout time).

Measurements

Hemodynamic, Respiratory, and Core Temperature Measurements. Heart rate was measured from the electrocardiogram. Mean arterial blood pressure, central venous pressure, mean pulmonary artery pressure, and pulmonary capillary wedge pressure were recorded with standard pressure transducers. All measurements except pulmonary capillary wedge pressure were displayed continuously on a multimodular monitor (S/5, Critical Care Monitor; GE Health Care, Chalfont St Giles, United King-
(ml · O₂/min). A thermodilution method was used to measure cardiac output every 30 min (the average of three measurements was calculated automatically by the monitor). Mixed venous oxygen saturation and hepatic vein oxygen saturation were continuously measured with fiberoptic catheters. Expired minute volume, tidal volume, respiratory rate, peak and other respiratory pressures, positive end-expiratory pressure, inspired and end-tidal carbon dioxide fraction, and inspired/expired oxygen fraction were monitored throughout the study. Core temperature was measured with a temperature probe incorporated in the pulmonary artery catheter.

Cardiac index (ml · kg⁻¹ · min⁻¹) and systemic vascular resistance index (mmHg · kg⁻¹ · min⁻¹) were indexed to body weight. Systemic vascular resistance index was calculated as: systemic vascular resistance index = (mean arterial pressure - central venous pressure)/cardiac index. Systemic oxygen delivery index (ml · kg⁻¹ · min⁻¹) and systemic oxygen consumption index (ml · kg⁻¹ · min⁻¹) were calculated using the following formulas: systemic oxygen delivery index = (cardiac index × arterial oxygen content); systemic oxygen consumption index = (cardiac index × (arterial – mixed venous oxygen content)). Oxygen content (ml · O₂/min blood) = ((arterial oxygen pressure × 0.0031) + (hemoglobin × arterial oxygen saturation × 1.36))/100.

**Blood Gas Measurements.** Arterial and mixed venous blood gas measurements were performed every 60 min. Oxygen pressure (P₀₂), carbon dioxide pressure (PₐCO₂), pH, lactate, oxygen saturation (SO₂), base excess, and total hemoglobin concentration (hemoglobin) were immediately measured with an analyzer designed for porcine blood (OSM 3; Radiometer, Copenhagen, Denmark) and with a human blood gas analyzer (ABL 520; Radiometer).

**Tissue Oxygen Tension Measurement.** For intestinal tissue oxygen tension measurement, the surgeon inserted the polarographic tissue oxygen tension sensors through a 20-gauge cannula into a section of healthy and of perianastomotic colon (2 cm proximally of the anastomosis) between the serosal and the mucosal tissue planes. This method has been previously described by several authors.22,33,39 Care was taken to minimize handling of the intestines and to return the bowels to a neutral position.

**Laser Doppler Flowmetry.** Laser Doppler flowmetry (LDF) probes were positioned on the muscularis and the mucosal side of healthy and perianastomotic colon. The signals of the LDF and the polarographic tissue oxygen tension sensors were visualized and recorded on a multichannel interface with a computer monitor via a multichannel interface with a sampling rate of 10 Hz (MP100; Biopac Systems, Goleta, CA) with acquisition software (Acqknowledge 3.9; Biopac Systems). If the signal quality of any probe was poor, the probe’s position was corrected immediately. Samples were averaged over 5-min intervals to obtain measurement values for analysis.

**Microdialysis.** Intestinal glucose, lactate, and pyruvate were measured using microdialysis probes (CMA/
Table 1. Continued

<table>
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<tr>
<th>Goal-directed Colloid (GD-C)</th>
<th>0 min</th>
<th>30 min</th>
<th>180 min</th>
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<td>57.2 ± 6.9</td>
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<td>3 ± 0.7</td>
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<td>14 ± 2</td>
<td>17 ± 3</td>
<td>16 ± 3</td>
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<td>116 ± 19#</td>
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<td>89 ± 11</td>
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<td>61.2 ± 2#</td>
<td>62.5 ± 3.5#</td>
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20). The microdialysis probe consisted of a polyethersulfone membrane with a molecular cut-off of 100,000 Dalton (probe size: length, 10 mm; OD, 0.5 mm). Before being used in vitro, the probes were flushed for 10 min with 70% ethanol to remove residual glycerol and for another 5 min with purified water to remove the ethanol. Before start of the experiment, the probe’s relative recovery of glucose, lactate, and pyruvate was established in vitro at 30°C with known concentrations of glucose (5.55 mM), lactate (2.5 mM), pyruvate (250 mM), and glycerol (475 mM) (Calibrator A; CMA Microdialysis). The probes were perfused at a constant flow rate of 1 μl · min⁻¹. After a period of 30 min, which is considered to be necessary to reach steady state equilibration, two samples were collected during fixed time intervals of 30 min. The recovery of the particular substance was then calculated as follows: Recovery in vitro = mean concentration dialysate × concentration standard solution⁻¹ × 100. During the study, the dialysate was collected in microvials for laboratory analysis. Dialysate was collected during the final 30 min of baseline and during treatment every 60 min for 30 min. Glucose, lactate, and pyruvate concentrations were measured using the CMA 600 system (CMA Microdialysis), which performs a spectrophotometric assay catalyzed by a kinetic enzymatic reaction for each parameter.

Wet/Dry Weight Ratio. Tissue samples were collected immediately after euthanasia for wet/dry weight ratio measurements from lung, healthy colon, and perianastomotic colon. Wet/dry ratio was measured with the formula: wet weight × freeze-dried weight⁻¹.

Statistical Methods
A power analysis was conducted as follows: Previous studies suggest a difference in tissue oxygen tension of more than 15 mmHg is clinically relevant. The SD of colonic tissue oxygen tension values in similar studies is typically approximately 15 mmHg. Assuming a difference of at least 20 mmHg between the three treatment groups, 9 pigs in each group provide an 80% power to detect a significant difference between the groups at an α-level of 0.05.

Before statistical analysis, data were tested for normality by QQ-plot and by Kolmogorow-Smirnow test. Baseline data were compared with analysis of variance (ANOVA) or Kruskall-Wallis test to exclude group discrepancies before start of treatment. Differences between the treatment groups for variables over time were assessed by ANOVA for repeated measurements with group as between-subject factor and time as within-subject factor. If a significant difference between the groups was detected, a post hoc test was performed to assess differences at individual time points. To account for multiple comparisons, a Tukey correction was employed. In addition, the area under the variable-time curve for each variable of interest was calculated and compared with ANOVA for group differences. Again a Tukey post hoc test was performed to compare individual treatments if the ANOVA had detected significant differences between the groups. As aforementioned, microcirculatory blood flow values (LDF) were transformed before statistical analysis so that baseline values were 100%. Similarly perianastomotic tissue oxygen tension values were transformed due to their large baseline variation. Absolute values were used for all other calculations. Data are presented as mean ± SD unless otherwise specified. P < 0.05 was considered significant. For statistical calculations, SAS Version 8 (SAS Institute Inc., Cary, NC) was used.

Results
All animals (n = 27; 9 animals per group) survived until the end of the treatment period. Animals in the low restricted crystalloid group received 924 ± 44 ml of RL during the entire study. Animals in the goal-directed crystalloid group received 943 ± 68 ml of RL plus 1794 ± 211 ml of RL as boluses of RL during the study. Animals in the colloid group received 917 ± 41 ml of RL plus 831 ± 267 ml as boluses of HES during the study. There were no differences in hemodynamic or metabolic variables for baseline measurements (Table 1, t = 0 min) between the three fluid groups.

Mixed Venous Oxygen Saturation, Systemic Hemodynamic Variables, Arterial Hemoglobin (Table 1) Mean mixed venous oxygen saturation was below the target value of Svo₂ of at least 60% in all three groups before start of treatment (baseline, t = 0 min). In the R-RL group, mean mixed venous saturation remained below 60% throughout the study (48.2 ± 3.9% after 4 h treatment). After the first fluid bolus, mixed venous oxygen saturation greater than 60% was reached in all animals in the GD-C group but in only one of nine animals in the GD-RL group. Until the end of the study,
six of nine animals had not reached the mixed venous saturation goal of 60% in the GD-RL group, despite repeated boluses.

Heart rate was lower in both goal-directed groups 
versus R-RL (P = 0.008). Mean arterial pressure was significantly higher in the GD-C versus the R-RL group (P = 0.007). Cardiac index differed in all groups (P < 0.001) and increased by 54 ± 17% in the GD-C, increased by 21 ± 11% in the GD-RL group, and decreased by 12 ± 7% in R-RL (percent of postoperative baseline). After 4 h of treatment, central venous pressure and mean pulmonary artery pressure were not different among the groups; in contrast, pulmonary capillary wedge pressure was higher in groups GD-C and GD-RL versus the R-RL group (P = 0.042). Systemic oxygen delivery (P < 0.001) and systemic oxygen extraction ratio (P < 0.001) increased significantly in both goal-directed groups versus the R-RL group. All groups had comparable arterial and mesenteric blood pH, PaO2, PaCO2, and lactate levels throughout the study. Arterial hemoglobin concentration increased in group R-RL and differed from group GD-C (P < 0.01).

Colon Tissue Oxygen Tension (Figs. 1A, B) and Microcirculatory Blood Flow in the Colon (Figs. 2A–D)

In healthy colon tissue, oxygen tension in group GD-C was higher in comparison to the R-RL group (P = 0.001). After 4 h of treatment, healthy colon tissue oxygen tension increased to 150 ± 31% in the GD-C group and to 123 ± 40% in the GD-RL group and decreased to 94 ± 23% in group R-RL (percent of postoperative baseline). After 4 h of treatment, perianastomotic tissue oxygen tension increased to 245 ± 93% in the GD-C group, to 147 ± 58% in the GD-RL, and to 116 ± 22% in group R-RL (P < 0.001).

Microcirculatory blood flow as measured by LDF in healthy colon mucosa increased only in the GD-C group, immediately after the first colloid bolus (P = 0.033). An increase was also measured in healthy colon muscularis (not significant). Microcirculatory blood flow did not differ among the groups in perianastomotic mucosal tissue. Microcirculatory blood flow in perianastomotic muscularis tissue was significantly higher in group GD-C compared with the GD-RL group (P = 0.042).

Intestinal Microdialysis Measurements, Regional Blood Gas

Colon or perianastomotic microdialysis measurements for tissue glucose or lactate/pyruvate ratios were not significantly different among the groups (fig. 3A–D).

Colon and Pulmonary Wet/Dry Weight Ratio

The wet/dry weight ratio of lung tissue was significantly higher in both the GD-RL and the GD-C group versus R-RL (P = 0.003) (fig. 4A–C). There were no differences among the groups in wet/dry weight ratio measurements in healthy or anastomotic colon tissue.

Discussion

This study demonstrates in a porcine model of open colon surgery that perioperative goal-directed administration of colloids markedly increases tissue oxygen tension and microcirculatory perfusion in healthy and perianastomotic colon. In the fluid-restricted group, colon tissue oxygen tension and perfusion remained at a lower level during the whole study, and both parameters increased slightly in the goal-directed crystalloid group. Interestingly, at the same time, there were no or only comparably small differences in hemodynamic parameters, i.e., heart rate, mean arterial pressure, central venous pressure, cardiac index, pulmonary capillary wedge pressure, and arterial lactate among the three treatment groups.
Several recent patient studies showed improved patient outcome after a goal-directed colloid fluid therapy in major surgery. The basic, tissue-level mechanisms, why the perioperative administration of colloid fluid had such a big impact on subsequent outcome, were not known. Our results help explain these findings; in the present study, the regional colloid fluid effects in injured, perianastomotic tissue were distinctly greater than systemic colloid effects. This supports the notion that improved patient outcome is primarily caused by improved perioperative intestinal microcirculatory blood flow and in-
creased tissue oxygen tension due to colloid fluid administration.

In accordance with the results of a previous study with fixed crystalloid administration,22 goal-directed administration of crystalloids had no pronounced effect on tissue oxygen tension and perfusion in perianastomotic and healthy colon. In the current study, we extended our previous abdominal surgical model22 with measurements of microcirculatory blood flow, microdialysis, and intestinal wet/dry ratio and included a goal-directed colloid group to allow a direct crystalloid-colloid comparison.

Interestingly, goal-directed colloid therapy increased perianastomotic tissue oxygen tension and serosal perfusion, but perianastomotic mucosal perfusion remained unchanged. The heterogeneity between serosal and mucosal tissue perfusion has already been documented by several authors.36,45 Mucosal tissue has distinct compensatory mechanisms, and mucosal flow is preserved by all means; we may thus suspect that perianastomotic mucosal flow was already exhausting its available compensatory mechanisms to maintain adequate perfusion. Consequently, perfusion could not be increased by fluid optimization with goal-directed colloids.

Microdialysis measurements in the gut are a fairly novel technique, and it has been hypothesized that the method may be used intraperitoneally in patients for early detection of intestinal tissue ischemia46 and anastomotic leaks.57 Our results are in accordance with previous reports on intestinal microdialysis in animal models.36,48 Krejci et al.36 found gut wall glucose to be an early marker of impaired intestinal perfusion; after a flow reduction of 30% in the mesenteric artery, intramural glucose concentration began to decrease. In the current study, we did not find significant differences for glucose content or lactate/pyruvate ratio between the groups in either healthy or perianastomotic colon tissue.

We may hypothesize that the hypoperfusion caused by the restricted fluid treatment was less than in the aforementioned study and thus caused no significant changes in gut wall glucose. In addition, it may be suggested that tissue oxygen tension and perfusion as measured by polarographic probes and Laser Doppler flow measurements are more sensitive methods to assess changes in tissue microcirculation than intramural intestinal microdialysis.

It has been shown previously that the administration of colloid fluids improves tissue oxygen tension in skeletal muscle of animal models49 and patients.50,51 However there are profound differences for the blood supply of skin, muscle, and colonic tissue, as has been shown, e.g., by the different effect of additional crystalloid fluid on subcutaneous tissue oxygen tension52 versus intestinal oxygen tension.22,35 Furthermore, it is not even appropriate to assume that perfusion and oxygenation changes in healthy and perianastomotic intestinal tissue occur in unison, as the results of our study indicate.

Why the colloid treatment had more beneficial effects on microcirculatory flow and tissue oxygenation in the current study is the result of many factors. It is likely that optimized global hemodynamics had some impact. In previous studies, hydroxyethyl starch has been shown to have complex beneficial effects on endothelial cells, inflammatory response, microvascular permeability, and rheology, e.g., in trauma,55 in ischemia reperfusion injury,54 and during sepsis.55 However, in the current study’s setting, we are not able to separate individual systemic and regional effects and are not able to conclude which factor is primarily responsible for the increase in tissue oxygen tension.

Tissue water content was measured in healthy, perianastomotic, and lung tissue with the wet/dry-ratio method. In septic animal models, the administration of
hydroxyethyl starch has been shown to inhibit capillary leakage and thus prevent lung edema and decrease lung tissue water content. In contrast, in the lung tissue of our nonseptic animals there was no difference for pulmonary wet/dry ratio between crystalloid or colloid administration, whereas the wet/dry ratios of both the GD-C and GD-RL animals were significantly higher in comparison to the restricted fluid group; albeit the administration was rather small. Surprisingly, there were no differences of wet/dry ratio among the groups in healthy or perianastomotic colon tissue. These results suggest that neither treatment markedly influenced intestinal edema, yet caution should be exercised during goal-directed fluid therapy in patients susceptible to lung edema, as wet/dry ratios were detected in the goal-directed groups.

This study has some limitations. Stress hormone levels as a response to possible hypovolemia were not measured. The study used many very invasive measurement methods not feasible for patient or volunteer use. Consequently, we chose to conduct the study in a porcine model, as the porcine intestinal system closely approximates the human intestinal system. Consistent with this theory, porcine subcutaneous and colonic tissue oxygen tension were comparable to values observed in humans. As “goal” for our goal-directed therapy, mixed venous saturation greater than 60% was used according to a previous study by Pearse et al. In the study, Pearse et al. measured central venous saturation, which is usually 4% higher than mixed venous saturation, and determined a cutoff of 64.4% for a significant reduction in postoperative complications and length of stay. Obviously, other goals could have been considered, like difference in pulse pressure, stroke volume variation, or corrected flow time as measured by esophageal Doppler.

Another limitation is the relatively short observation period after surgery (4 h). However, previous authors have suggested that the immediate perioperative period constitutes the decisive hours for the later development of wound infection or leakage and thus merits a special focus. Finally, it is important to keep in mind that all experimental animals were young and healthy, which is in contrast to the clinical reality, where most patients have one or more concomitant diseases. We tried to imitate reduced ability to compensate a surgical hit by artificially deteriorating anastomotic conditions with additional perianastomotic blood supply ligations.

In conclusion, goal-directed crystalloid therapy and restricted fluid therapy did not change healthy or perianastomotic colon tissue microcirculation. In contrast, goal-directed colloid therapy considerably increased oxygen tension and perfusion in healthy and injured colon tissue.

References


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