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A RANDOMIZED PORCINE STUDY IN LOW CARDIAC OUTPUT OF VASOACTIVE AND INOTROPIC DRUG EFFECTS ON THE GASTROINTESTINAL TRACT

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ABSTRACT—**Background:** Splanchnic vasodilation by inodilators is an argument for their use in critical cardiac dysfunction. To isolate peripheral vasoactivity from inotropy, such drugs were investigated, and contrasted to vasopressors, in a fixed low cardiac output (CO) model resembling acute cardiac dysfunction effects on the gastrointestinal tract. We hypothesized that inodilators would vasodilate and preserve the aerobic metabolism in the splanchnic circulation in low CO. **Methods:** In anesthetized pigs, CO was lowered to 60% of baseline by partial inferior caval vein balloon inflation. The animals were randomized to placebo (n = 8), levosimendan (24 $\mu g\,kg^{-1}$ bolus, 0.2 $\mu g\,kg^{-1}\,min^{-1}$, n = 7), milrinone (50 $\mu g\,kg^{-1}$ bolus, 0.5 $\mu g\,kg^{-1}\,min^{-1}$, n = 7), vasopressin (0.001, 0.002 and 0.006 U $kg^{-1}\,min^{-1}$, 1 h each, n = 7) or norepinephrine (0.04, 0.12, and 0.36 $\mu g\,kg^{-1}\,min^{-1}$, 1 h each, n = 7). Hemodynamic variables including mesenteric blood flow were collected. Systemic, mixed-venous, mesenteric-venous, and intraperitoneal metabolites were analyzed. **Results:** Cardiac output was stable at 60% in all groups, which resulted in systemic hypotension, low superior mesenteric artery blood flow, lactic acidosis, and increased intraperitoneal concentrations of lactate. Levosimendan and milrinone did not change any circulatory variables, but levosimendan increased blood lactate concentrations. Vasopressin and norepinephrine increased systemic and mesenteric vascular resistances at the highest dose. Vasopressin increased mesenteric resistance more than systemic, and the intraperitoneal lactate concentration and lactate/pyruvate ratio. **Conclusion:** Splanchnic vasodilation by levosimendan and milrinone may be negligible in low CO, thus rejecting the hypothesis. High-dose vasopressors may have side effects in the splanchnic circulation.

KEYWORDS—Cardiovascular agents, catecholamines, intraperitoneal microdialysis, phosphodiesterase inhibitors, splanchnic circulation

INTRODUCTION

Circulatory compromise may affect the splanchnic circulation, leading to the loss of intestinal mucosal barrier function and bacterial translocation (1). This may in turn initiate, maintain, or aggravate a systemic inflammatory reaction (2). The number of gastrointestinal symptoms has been demonstrated to independently predict 28-day mortality during intensive care (3), showing the importance of maintaining gastrointestinal function and integrity in the critically ill. Drugs used to treat low cardiac output (CO) and/or manipulate the vascular resistance are often required (4). These potent drugs have the potential to both promote and impair splanchnic perfusion, but since the pathophysiological mechanisms between conditions such as hemorrhagic, septic and cardiogenic shock differ, the results from one condition may not be directly transferable to another (5). It has been shown that the peripheral effects of inodilators can be advantageous for renal function when used in low CO (6, 7). Thus, clinical rationale to use inodilators in acute cardiac dysfunction may include both improvement of cardiac function and beneficial peripheral vasodilation (8, 9). However, global measures of circulation, e.g., systemic blood pressure and CO, are not enough to ensure that the selected therapy has the intended effect on splanchnic perfusion (5). Furthermore, when exploring potential circulatory regional organ effects of inodilators, previous experimental studies have mainly investigated the combination of inotropic and vasodilatory effects (10, 11), and cannot differentiate the magnitude and clinical importance of the respective effect. Especially, it is unknown whether the increased splanchnic blood flow by inodilators is due to enhanced CO or direct splanchnic vasodilation. Similarly, alpha- and beta-adrenergic agents such as norepinephrine may both have cardiac contractile and direct peripheral vasoconstrictive effects. In comparison, a simple calculation of pulmonary vascular resistance falsely indicated, due to increased CO, that norepinephrine was a pulmonary vasodilator in pulmonary embolism; however, a detailed analysis using pressure-flow plots excluded effects of

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norepinephrine on pulmonary vascular tone in this condition (12). To determine the pharmacological effect of inodilators and vasoconstrictors on vessel resistance independent of inotropic influences, which is of clinical interest, arguably a controlled CO model must be used.

The primary aim of this study was to investigate the effects of inodilators (levosimendan and milrinone) on gastrointestinal circulation and metabolism in a porcine model of fixed low CO to isolate peripheral vasoactivity, and the secondary aim was to contrast these drugs to the effects of vasopressors (vasopressin and norepinephrine). The hypothesis was that the inodilators would have vasodilatory effects on the splanchnic circulation and therefore cause increased gastrointestinal blood flow and preserved aerobic metabolism, while the vasopressors would have the opposite effects.

MATERIALS AND METHODS

Animals

The experiments were approved by the Regional Animal Research Ethics Committee, Linköping, Sweden (reference number 38-15) and conducted in accordance with the guidelines of the European Union for the protection of animals used for scientific purposes (13). Thirty-nine healthy, 3-month-old, domestic pigs (a crossbreed between Swedish country breed, Hampshire, and Yorkshire) of both sexes and with a mean body weight of 25 kg (range 20 kg–29 kg) were used in this study. The animals had free access to water and standard porcine fodder prior to the experiments and were housed indoors at room temperature in large pens with 10 L in each. They were kept in a 16-h day and 8-h night cycle.

Anesthesia and surgical preparation

Anesthesia, surgical preparation, and measurement techniques were similar to the method described in a previous study (14).

Throughout the experiment, anesthesia was maintained with propofol ($10\,mg\,kg^{-1}\,h^{-1}$–$15\,mg\,kg^{-1}\,h^{-1}$, i.v.) and fentanyl ($5\,\mu g\,kg^{-1}\,h^{-1}$–$20\,\mu g\,kg^{-1}\,1\,h^{-1}$, i.v.). Ringer acetate ($10\,mL\,kg^{-1}\,h^{-1}$, i.v.) and 10% glucose with 40 mM sodium and 20 mM potassium ($0.5\,mL\,kg^{-1}\,h^{-1}$, i.v.) were administered. After instrumentation 5,000 IU heparin i.v. was given. The pigs were orally intubated and ventilated in volume controlled mode. At baseline, the minute ventilation was set to arterial PCO_2 4.7 kPa to 5.5 kPa, and it was adjusted throughout the experiment to compensate for arterial acid–base deterioration. Arterial PO_2 was kept within the normal range by means of oxygen supplementation to inhaled gas. The positive-end expiratory pressure was set at 4 cmH_2O to 6 cmH_2O. Euthanasia was achieved with an i.v. injection of 40 mmol potassium chloride after the conclusion of the experiment.

The following catheters were inserted: a 7 Fr triple-lumen central line in the left external jugular vein (for fluid and drug infusion), a 4 Fr introducer in the right carotid artery (for pressure measurements and arterial blood sampling), an 8 Fr introducer (for a pulmonary artery catheter used for semicontinuous CO measurements [Swan-Ganz CCOmbo, 7.5 Fr, Edwards Lifesciences, Irvine, Calif] and mixed venous blood sampling) and an 11 Fr introducer (for a balloon catheter placed in the diaphragmatic (suprahepatic) part of the inferior caval vein [Cordis PTA Dilatation Catheter, large diameter balloon, 7 Fr, 80 cm, Cordis Corporation, Miami Lakes, Fla]) in the right external jugular vein. After a midline abdominal incision, a 14 Fr Foley catheter was positioned in the urinary bladder, an ultrasonic transit-time flowmeter (Vascular TTFM Probe 6 mm, Medistim ASA, Oslo, Norway) was put around the superior mesenteric artery (SMA), and a 6 Fr catheter (Nutrisafe 2, Vygon, Ecouen, France) was inserted in the superior mesenteric vein for pressure measurement and mesenteric venous blood sampling. Finally, a free-floating microdialysis catheter (62 Gastrointestinal Microdialysis Catheter, Membrane length 30 mm, cutoff 20 kDa, M Dialysis, Stockholm, Sweden), connected to a microdialysis pump (107 Microdialysis Pump, Perfusion fluid T1 at $2\,\mu L\,min^{-1}$, M Dialysis), was placed intraperitoneally in the left lower quadrant.

Blood samples were analyzed at 37°C for pH, blood gases, electrolytes, lactate, hemoglobin (Hb) and glucose using GEM Premier 4, 000 (Instrumentation Laboratory, Lexington, Mass). The microdialysate was analyzed for glucose, glycerol, lactate, and pyruvate using a designated analyzer (CMA 600, M Dialysis).

Protocol

After instrumentation, the animals were rested for half an hour to achieve normal body temperature and stable variables. This was followed by 1 h of observation to establish baseline values. Cardiac output was then lowered and maintained at 60% of the baseline value for 4 h by partial inflation of the inferior caval vein balloon. Cardiac output was semicontinuously monitored with updates at least every 60 s, and adjustments in balloon inflation to maintain the targeted CO were made every 15 min. Positioning and inflation of the balloon were confirmed by fluoroscopy. After 1 h of low CO, the animals were randomized to one of five groups: placebo (5% glucose, Fresenius Kabi, Uppsala, Sweden; $0.2\,mL\,kg^{-1}\,h^{-1}$), levosimendan (Simdax, Orion Pharma, Sollentuna, Sweden; bolus $24\,\mu g\,kg^{-1}$ in 10 min, thereafter continuous infusion of $0.2\,\mu g\,kg^{-1}\,min^{-1}$), milrinone (Corotrop, Sanofi, Stockholm, Sweden; bolus $50\,\mu g\,kg^{-1}$ in 10 min, thereafter continuous infusion of $0.5\,\mu g\,kg^{-1}\,min^{-1}$), vasopressin (Pitressin; Daiichi-Sankyo Co, Japan; $0.001\,U\,kg^{-1}\,min^{-1}$ for the first hour, $0.002\,U\,kg^{-1}\,min^{-1}$ for the second hour, and $0.006\,U\,kg^{-1}\,min^{-1}$ for the last hour), and norepinephrine (Noradrenalin, Hospira Nordic, Stockholm, Sweden; $0.04\,\mu g\,kg^{-1}\,min^{-1}$ for the first hour, $0.12\,\mu g\,kg^{-1}\,min^{-1}$ for the second hour, and $0.36\,\mu g\,kg^{-1}\,min^{-1}$ for the last hour). The protocols for administration were chosen based on the pharmacokinetics of each drug. Levosimendan and milrinone were administered as a bolus followed by a constant infusion, with known contractile effects in pigs, due to their long half-live in the circulation (15, 16). The investigation of levosimendan and milrinone is not a dose–response experiment but a comparison over time with placebo. Norepinephrine and vasopressin were administered in three doses (low, middle, and high-very high needed for a dose–response increment in systemic blood pressure in pigs) in a cumulative manner, due to their fast on-response and short half-lives in the circulation (17, 18). Each dose was administered for 1 h to be able to detect any metabolic effects, and the comparison against a placebo group was necessary due to the potential dynamic of a low CO model. The aim of the study design was not to compare inodilators with vasopressors. Importantly, the dosage and protocols of the drugs are similar to clinically suggested in low CO syndrome, except the highest dose of vasopressin (4).

Measurements were performed at baseline and then at hourly intervals for 4 h. The microdialysate was collected during the last 30 min of each hour. Animals that died before randomization were excluded from the study. Animals that died prematurely but after randomization or those that developed severe hypoglycemia (plasma glucose concentration $\leq$ 1.5 mM) and/or severe systemic hypotension (mean arterial pressure [MAP] $<$ 25 mm Hg) were excluded at that time point, although data sampled before that time point were used.

Calculations

Cardiac output and SMA blood flow were divided by body surface area, according to the formula: body surface area $(m^2) = 0.0734 \times \text{weight (kg)}^{0.656}$, to obtain the cardiac index (CI) and the SMA blood flow index (19). Porcine Hb oxygen saturation was calculated as $PO_2^{2.94}/(PO_2^{2.94} + P_{50}^{2.94})$, since the blood gas machine was calibrated for human oximetry. 4.76 kPa was used as the porcine partial PO_2, where Hb is 50% saturated (P_{50}), and the value was adjusted with the fixed acid Bohr coefficient (20). Oxygen content was calculated as Saturation (fraction) $\times$ Hb (g/L) $\times$ 1.34 + 0.225 $\times$ PO_2 (kPa) (21). Oxygen consumption was calculated as oxygen extraction $\times$ blood flow index. The mesenteric vascular resistance index (MVRI) was calculated as (MAP − mesenteric venous pressure [MVP])/SMA blood flow index. The systemic vascular resistance index (SVRI) was calculated as (MAP − central venous pressure [CVP])/CI.

Statistical analysis

Approximate normal distribution and equal variances were analyzed using Shapiro–Wilk test and Levene test, respectively. Logarithmic transformation was used for some variables (intraperitoneal glycerol and lactate concentrations) to achieve normal distribution. An *a priori* sample size calculation was not done due to the lack of preliminary data from pilot experiments or published data in a similar model with these drugs. Main (time and group) and simple (the interaction between time and group) fixed effects were analyzed using a linear mixed model with a homogenous (if equal variances) or heterogeneous (if nonequal variances) autoregressive covariance structure. In the linear mixed model analysis, animals excluded late in the experiments were included in the model (Fig. 1). A Bonferroni adjusted *post hoc* multiple comparison was then performed if fixed effects (time or interaction between time and group) were significant. In the *post hoc* multiple comparison between the groups at the various time points, data from all animals not excluded at the respective time points were included in the analysis, see Figure 1 for the exact number of animals. A P value of less than 0.05 was regarded as statistically significant. Data are presented as means and 95% confidence intervals. Statistical analysis was performed using IBM SPSS Statistics version 25.0 for Windows (SPSS Inc, Chicago, Ill).

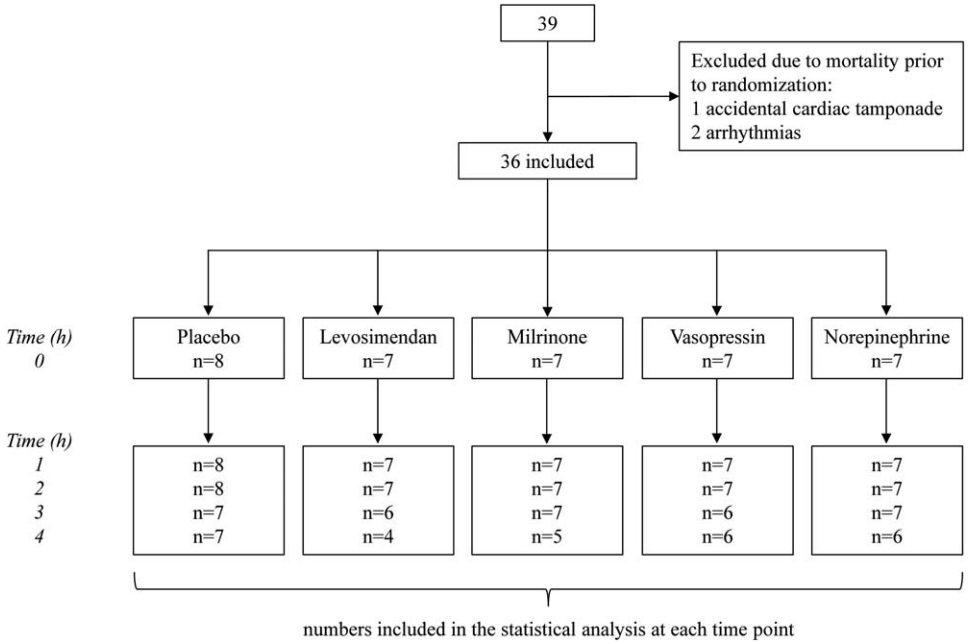

Fig. 1. **Animal inclusion by randomization and animal exclusions in the different study groups.**

RESULTS

Thirty-six of 39 pigs were included. Three pigs were excluded from the study before randomization due to early mortality. After randomization, eight animals were excluded due to low plasma glucose levels or death but were included in the statistical analyses up to exclusion (Fig. 1).

During the experiments, the animals maintained a normal body temperature and were normoxemic (data not shown). The minute ventilation was increased to partially compensate for the metabolic acidosis, resulting in slightly decreased arterial PCO_2 (data not shown, but all animals had arterial $PCO_2 > 3.8$ kPa at all measurements).

Cardiac index

Through inflation of the inferior caval vein balloon, the CI significantly decreased and was maintained at 60% of baseline value in all groups, with no significant differences between the groups (Fig. 2). Only minor inflations or deflations of the balloon were necessary to keep a stable CI throughout the experiments after the initial inflation (median volume in the balloon at the first, second, third, and last hours was 3.4 mL, 3.3 mL, 3.2 mL, and 3.0 mL, respectively).

Effects of low cardiac output

In the placebo group, in parallel with the decrease in CO, MAP, and CVP decreased significantly, and heart rate, SVRI, and MVP increased significantly (Fig. 3 and Table 1). At low CO, the blood flow in SMA decreased significantly whereas MVRI and the MVRI/SVRI ratio were unchanged (Fig. 3). Arterial pH was significantly decreased after the first hour of low CO (Table 1). The arterial, mixed venous, mesenteric venous, and intraperitoneal lactate concentrations were significantly increased compared with baseline after the first hour of low CO, whereas the arterial to mesenteric venous lactate concentration difference and intraperitoneal lactate/pyruvate ratio were unchanged (Table 1). After the second hour of low CO, the intraperitoneal glycerol concentration was significantly increased (Table 1). Systemic and mesenteric oxygen consumptions were maintained at low CO (Table 1). There were no statistically significant differences between the placebo group and the treatment groups at baseline and 1 h, i.e., before the start of drug infusions.

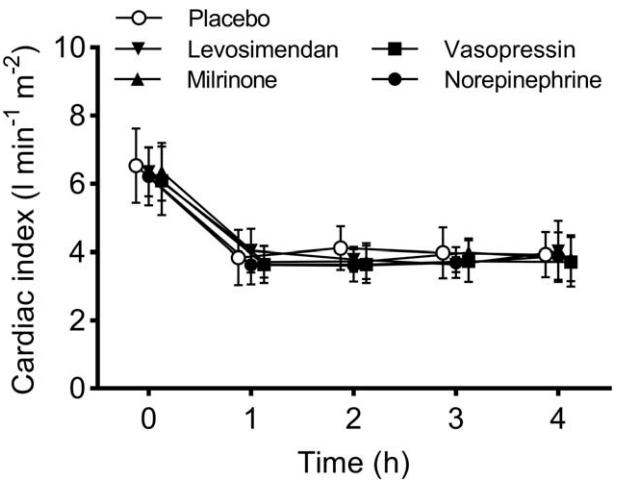

Fig. 2. **Cardiac indices in animals subjected to partial inflation of an occlusion balloon in the inferior caval vein immediately after time point 0 h.** Starting at 1 h, the animals were randomized to intravenous infusion of either placebo (5% glucose, 0.2 mL kg^{-1} h^{-1}), vasopressin (0.001 U kg^{-1} min^{-1} for the first hour, 0.002 U kg^{-1} min^{-1} for the second hour, and 0.006 U kg^{-1} min^{-1} for the last hour), norepinephrine (0.04 μg kg^{-1} min^{-1} for the first hour, 0.12 μg kg^{-1} min^{-1} for the second hour, and 0.36 μg kg^{-1} min^{-1} for the last hour), levosimendan (bolus 24 μg kg^{-1} in 10 min, thereafter continuous infusion of 0.2 μg kg^{-1} min^{-1}) or milrinone (bolus 50 μg kg^{-1} in 10 min, thereafter continuous infusion of 0.5 μg kg^{-1} min^{-1}). Data are presented as means and 95% confidence intervals. See Figure 1 for the exact number of animals in each group at each time point.

The inodilators

None of the hemodynamic variables were significantly affected by levosimendan or milrinone (Fig. 3). Arterial, mixed venous, and mesenteric venous lactate concentrations were significantly higher in the levosimendan group compared with the placebo group, whereas arterial pH was similar and changes in the intraperitoneal lactate concentration and lactate/pyruvate ratio did not reach statistical significance (Table 1). Milrinone did not significantly change the arterial pH or lactate concentrations in any location compared with placebo (Table 1). Systemic and mesenteric oxygen consumption were not affected by the inodilators (Table 1). In both inodilator groups, intraperitoneal glycerol concentrations were higher at the final hourly measurement compared with the placebo group (Table 1).

The vasopressors

At the highest dose, vasopressin and norepinephrine increased MAP, SVRI, and MVRI to values that were significantly higher than in the placebo group (Fig. 4). The vasopressors did not affect the blood flow in SMA, CVP, or MVP (Fig. 4 and Table 1). The MVRI/SVRI ratio was significantly higher and heart rate significantly lower at the highest vasopressin dose compared with placebo, whereas norepinephrine did not affect these measures (Fig. 4). At the highest dose of vasopressin, intraperitoneal lactate concentration and lactate/pyruvate ratio were higher than in the placebo group, whereas these variables were similar in the norepinephrine and the placebo groups (Table 1). The vasopressors did not significantly change arterial pH or lactate concentrations, intraperitoneal glycerol concentrations, or systemic and mesenteric oxygen consumption (Table 1).

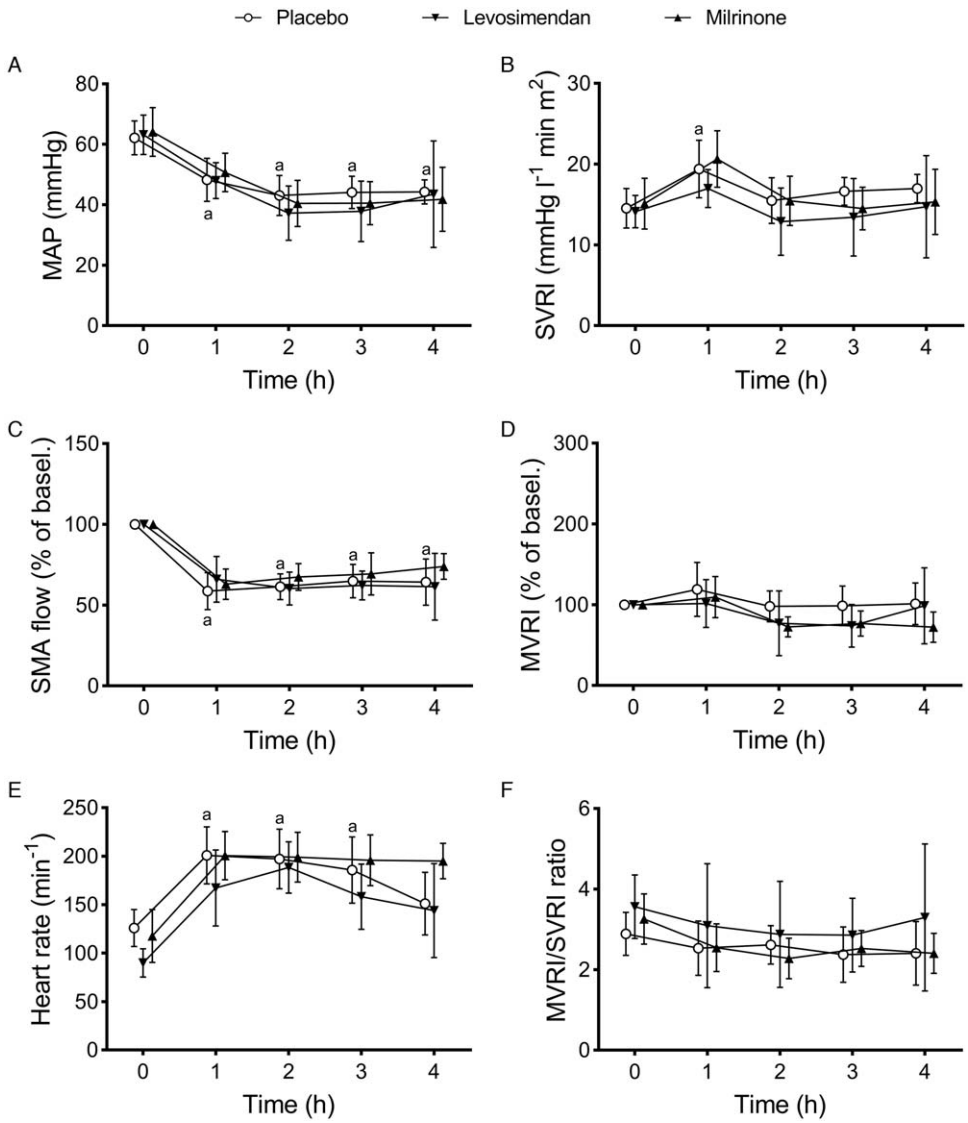

Fig. 3. **Mean arterial pressure (MAP, panel A), mesenteric and systemic vascular resistance indices, and their ratio (MVRI, panel D; SVRI, panel B; MVRI/SVRI ratio, panel F), superior mesenteric artery blood flow (SMA flow, panel C), and heart rate (panel E) in animals subjected to partial inflation of an occlusion balloon in the inferior caval vein immediately after time point 0 h.** Starting at 1 h, the animals were randomized to intravenous infusion of either placebo (5% glucose, 0.2 mL kg^{-1} h^{-1}), levosimendan (bolus 24 μg kg^{-1} in 10 min, thereafter continuous infusion of 0.2 μg kg^{-1} min^{-1}) or milrinone (bolus 50 μg kg^{-1} in 10 min, thereafter continuous infusion of 0.5 μg kg^{-1} min^{-1}). [a]: statistical difference between the indicated time point and baseline in the placebo group. Data are presented as means and 95% confidence intervals. See Figure 1 for the exact number of animals in each group at each time point.

Table 1. **Measurements in animals subjected to partial inflation of an occlusion balloon in the inferior caval vein, immediately after the time point 0 h, to achieve a fixed cardiac output reduction to 60% of the baseline value.**

Variable	Time (h)	Placebo	Levosimendan	Milrinone	Vasopressin	Norepinephrine
Central venous pressure (mm Hg)	0	7 (5–8)	9 (8–11)	8 (6–9)	8 (6–9)	6 (4–7)
	1	5 (3–6) *	7 (5–9)	5 (4–7)	6 (4–7)	4 (3–6)
	2	5 (4–7)	7 (6–9)	6 (4–7)	6 (4–7)	5 (4–7)
	3	5 (4–7)	7 (6–9)	6 (4–7)	6 (5–8)	5 (3–6)
	4	5 (4–7)	7 (6–9)	6 (4–7)	7 (6–9)	5 (3–6)
Mesenteric venous pressure (mm Hg)	0	11 (9–12)	12 (11–13)	11 (10–13)	11 (10–13)	11 (9–12)
	1	14 (12–15) *	16 (14–17)	15 (14–17)	16 (14–17)	14 (13–16)
	2	12 (11–14)	15 (14–16)	15 (13–16)	15 (13–16)	13 (12–15)
	3	12 (11–13)	14 (13–16)	13 (12–15)	15 (13–16)	13 (11–14)
	4	12 (11–14)	13 (11–15)	13 (11–14)	13 (11–14)	13 (11–14)
Global oxygen consumption ($mL\ O_2\ m^{-2}\ min^{-1}$)	0	301 (267–336)	317 (282–353)	327 (291–363)	295 (259–331)	277 (241–313)
	1	311 (277–344)	330 (294–366)	324 (288–360)	323 (287–359)	298 (262–334)
	2	327 (294–360)	303 (268–338)	313 (278–348)	327 (292–362)	283 (248–318)
	3	320 (282–359)	275 (234–316)	336 (296–376)	304 (263–346)	298 (257–339)
	4	312 (265–359)	271 (214–328)	294 (242–347)	276 (225–326)	286 (235–337)
Mesenteric oxygen consumption ($mL\ O_2\ m^{-2}\ min^{-1}$)	0	57 (47–68)	56 (45–67)	58 (46–69)	46 (34–57)	51 (40–62)
	1	58 (48–69)	63 (51–74)	60 (49–71)	54 (43–66)	55 (44–66)
	2	58 (47–68)	54 (43–65)	54 (43–66)	51 (40–63)	53 (42–64)
	3	54 (43–65)	51 (39–62)	56 (44–67)	49 (37–60)	51 (39–62)
	4	55 (44–66)	42 (30–55)	51 (39–63)	41 (29–53)	50 (38–61)
Arterial pH	0	7.48 (7.43–7.52)	7.51 (7.46–7.55)	7.49 (7.44–7.54)	7.53 (7.48–7.58)	7.48 (7.44–7.53)
	1	7.38 (7.33–7.42) *	7.37 (7.32–7.42)	7.37 (7.32–7.41)	7.39 (7.35–7.44)	7.40 (7.35–7.45)
	2	7.42 (7.37–7.46)	7.35 (7.30–7.40)	7.38 (7.33–7.43)	7.41 (7.36–7.46)	7.40 (7.35–7.45)
	3	7.46 (7.42–7.51)	7.39 (7.34–7.44)	7.41 (7.36–7.46)	7.46 (7.41–7.51)	7.42 (7.38–7.47)
	4	7.49 (7.44–7.54)	7.50 (7.44–7.56)	7.44 (7.38–7.49)	7.51 (7.45–7.56)	7.45 (7.40–7.50)
Arterial glucose concentration (mM)	0	6.1 (5.1–7–1)	5.7 (5.4–6.1)	5.6 (5.2–6.0)	5.5 (5.2–5.8)	5.9 (5.1–6.7)
	1	7.1 (5.8–8.4)	7.7 (4.6–10,8)	6.5 (5.7–7.3)	6.3 (5.6–7.0)	7.4 (4.8–10.1)
	2	5.2 (4.6–5.9)	7.2 (4.3–10.1)	4.9 (3.5–6.3)	5.3 (4.9–5.8)	6.6 (3.7–9.4)
	3	4.7 (4.2–5.3)	5.2 (3.4–7.1)	4.4 (3.3–5.5)	4.8 (4.4–5.2)	5.6 (3.8–7.4)
	4	4.4 (4.0–4.8)	4.8 (3.7–5.9)	4.3 (3.4–5.3)	3.6 (3.0–4.3)	5.5 (4.8–6.1)
Arterial lactate concentration (mM)	0	2.8 (1.9–3.6)	1.9 (1.0–2.8)	2.2 (1.3–3.1)	2.0 (1.1–2.9)	2.4 (1.4–3.3)
	1	5.0 (3.1–6.9) *	5.8 (3.8–7.8)	5.8 (3.8–7.8)	4.8 (2.8–6.8)	4.7 (2.7–6.7)
	2	4.4 (2.5–6.3)	7.3 (5.2–9.3)	6.2 (4.1–8.2)	4.8 (2.8–6.9)	4.9 (2.9–7.0)
	3	3.4 (1.5–5.2)	7.4 (5.5–9.4) †	5.2 (3.3–7.1)	4.2 (2.2–6.1)	4.5 (2.6–6.4)
	4	2.7 (0.9–4.4)	6.6 (4.6–8.6) †	4.7 (2.8–6.6)	3.8 (1.9–5.7)	3.8 (1.9–5.6)
Mixed venous lactate concentration (mM)	0	2.8 (2.0–3.6)	1.9 (1.6–2.2)	2.2 (1.8–2.7)	2.0 (1.4–2.6)	2.4 (1.9–3.0)
	1	5.1 (2.8–7.3) *	5.8 (4.8–6.9)	5.8 (4.4–7.2)	5.0 (3.4–6.5)	4.9 (3.3–6.4)
	2	4.5 (2.0–7.0)	7.3 (5.4–9.2)	6.2 (3.7–8.7)	4.9 (3.2–6.5)	4.9 (2.6–7.2)
	3	2.5 (1.8–3.3)	7.4 (4.4–10.4) †	5.3 (2.4–8.2)	3.7 (2.4–5.0)	4.0 (2.3–5.7)
	4	2.0 (1.6–2.5)	5.5 (1.1–10.0) †	3.7 (1.4–5.9)	3.4 (2.0–4.8)	3.2 (1.6–4.8)
Mesenteric venous lactate concentration (mM)	0	2.7 (1.9–3.5)	1.9 (1.1–2.8)	2.2 (1.4–3.0)	2.1 (1.3–3.0)	2.5 (1.7–3.3)
	1	5.2 (3.0–7.3) *	5.5 (3.3–7.8)	5.8 (3.6–8.1)	4.7 (2.4–6.9)	4.9 (2.7–7.2)
	2	4.5 (2.4–6.6)	7.4 (5.1–9.6)	6.2 (4.0–8.4)	4.8 (2.6–7.0)	5.2 (2.9–7.4)
	3	3.5 (1.4–5.6)	7.8 (5.5–10.0) †	5.4 (3.2–7.6)	4.4 (2.1–6.6)	4.8 (2.6–7.0)
	4	2.8 (1.0–4.7)	6.9 (4.9–9.0) †	4.6 (2.6–6.6)	4.3 (2.4–6.3)	3.9 (2.0–5.9)
Arterial to mesenteric lactate difference (mM)	0	0.1 (−0.2 to 0.3)	−0.1 (−0.3 to 0.2)	0.0 (−0.3 to 0.2)	−0.1 (−0.3 to 0.1)	−0.1 (−0.4 to 0.1)
	1	−0.2 (−0.7 to 0.3)	0.2 (−0.3 to 0.8)	−0.1 (−0.6 to 0.5)	0.2 (−0.3 to 0.7)	−0.2 (−0.7 to 0.3)
	2	−0.1 (−0.5 to 0.3)	−0.1 (−0.5 to 0.3)	−0.1 (−0.4 to 0.3)	0.1 (−0.3 to 0.4)	−0.2 (−0.6 to 0.1)
	3	−0.1 (−0.4 to 0.3)	−0.3 (−0.7 to 0.0)	−0.2 (−0.6 to 0.1)	−0.2 (−0.6 to 0.2)	−0.3 (−0.7 to 0.1)
	4	−0.2 (−0.5 to 0.1)	−0.4 (−0.8 to 0.0)	0.0 (−0.3 to 0.4)	−0.6 (−0.9 to −0.2)	−0.2 (−0.5 to 0.2)
Intraperitoneal lactate concentration (mM)	0	4.6 (3.8–5.5)	3.5 (2.9–4.3)	4.8 (3.8–5.9)	4.0 (3.0–5.4)	4.2 (3.1–5.8)
	1	6.3 (5.1–7.7) *	6.7 (5.8–7.8)	7.3 (6.7–8.0)	6.3 (5.1–7.8)	5.7 (5.1–6.3)
	2	6.8 (5.1–9.2) *	8.8 (6.9–11.1)	8.7 (6.3–11.9)	7.1 (6.2–8.2)	6.8 (5.7–8.2)
	3	5.1 (4.3–6.1)	9.1 (7.0–11.8)	7.3 (4.5–11.8)	7.0 (6.1–8.0)	6.6 (5.1–8.5)
	4	4.3 (3.5–5.4)	7.4 (4.4–12.5)	6.7 (3.6–12.3)	7.3 (6.2–8.5) †	6.0 (4.5–8.1)
Intraperitoneal pyruvate concentration (μM)	0	261 (229–293)	281 (247–315)	315 (244–387)	272 (205–340)	317 (232–402)
	1	315 (250–381)	383 (291–475)	369 (303–436)	329 (274–383)	343 (270–415)
	2	331 (244–419)	430 (312–549)	377 (226–529)	343 (308–378)	383 (311–455)
	3	268 (212–324)	407 (310–504)	338 (167–508)	299 (248–351)	356 (294–418)
	4	255 (208–303)	340 (204–475)	290 (114–465)	315 (243–388)	353 (252–454)
Intraperitoneal lactate/pyruvate ratio	0	18.7 (14.7–22.7)	13.0 (10.8–15.1)	17.0 (11.8–22.2)	15.8 (13.0–18.5)	14.2 (12.6–15.8)
	1	21.0 (17.5–24.5)	18.9 (15.4–22.4)	20.6 (17.5–23.7)	20.1 (16.2–24.0)	20.3 (17.7–22.9)
	2	22.4 (17.8–27.1)	22.5 (17.7–27.3)	27.0 (20.5–33.4)	21.5 (17.7–25.3)	18.6 (13.5–23.6)
	3	20.8 (15.3–26.3)	24.4 (17.8–31.0)	26.9 (21.2–32.5)	24.5 (19.6–29.5)	17.8 (15.1–20.5)
	4	17.8 (14.5–21.1)	25.4 (13.8–37.0)	29.4 (18.9–39.9)	25.0 (18.6–31.3) †	18.0 (16.9–19.0)
Intraperitoneal glycerol concentration (μM)	0	78 (57–107)	52 (29–93)	75 (44–127)	55 (34–89)	65 (38–112)
	1	104 (68–159)	79 (56–111)	113 (80–160)	67 (46–98)	104 (76–144)
	2	140 (71–275) *	194 (112–335)	224 (138–364)	118 (91–153)	142 (89–225)
	3	75 (46–124)	232 (117–461)	256 (127–517)	102 (58–178)	137 (77–241)
	4	51 (28–93)	171 (44–660)†	166 (66–418)†	80 (41–156)	125 (66–235)

Table 1. (***continued***)

Variable	Time (h)	Placebo	Levosimendan	Milrinone	Vasopressin	Norepinephrine
Intraperitoneal glucose concentration (mM)	0	3.8 (2.9–4.8)	4.2 (3.0–5.3)	3.8 (2.5–5.1)	3.5 (2.8–4.2)	4.7 (3.6–5.8)
	1	3.5 (2.8–4.1)	4.7 (2.2–7.2)	2.9 (2.1–3.7)	3.0 (2.3–3.6)	4.1 (2.3–5.9)
	2	3.1 (2.3–4.0)	4.8 (1.1–8.4)	2.5 (1.0–4.0)	2.7 (1.7–3.7)	4.4 (2.2–6.6)
	3	2.2 (1.1–3.2) *	3.6 (0.9–6.3)	2.1 (1.0–3.1)	1.7 (0.6–2.8)	3.6 (1.6–5.5)
	4	2.3 (1.5–3.1)	3.0 (0.3–5.7)	1.5 (0.2–2.9)	1.3 (0.5–2.1)	3.8 (2.1–5.5)

Intravenous infusion started at 1 h. Placebo was 5% glucose at 0.2 mL kg^{-1} h^{-1}. Vasopressin was given as 0.001 U kg^{-1} min^{-1} for the first hour, 0.002 U kg^{-1} min^{-1} for the second hour, and 0.006 U kg^{-1} min^{-1} for the last hour. Norepinephrine was administered as 0.04 μg kg^{-1} min^{-1} for the first hour, 0.12 μg kg^{-1} min^{-1} for the second hour, and 0.36 μg kg^{-1} min^{-1} for the last hour. Levosimendan was given as a bolus of 24 μg kg^{-1} in 10 min, thereafter continuous infusion of 0.2 μg kg^{-1} min^{-1}. Milrinone was administered as a bolus of 50 μg kg^{-1} in 10 min, thereafter continuous infusion of 0.5 μg kg^{-1} min^{-1}.

*Statistically significant difference between baseline and the indicated time point in the placebo group.

†Statistically significant difference between the placebo group and one of the treatment groups at the indicated time points. Data are means and 95% confidence intervals. See Figure 1 for the exact number of animals in each group at each time point.

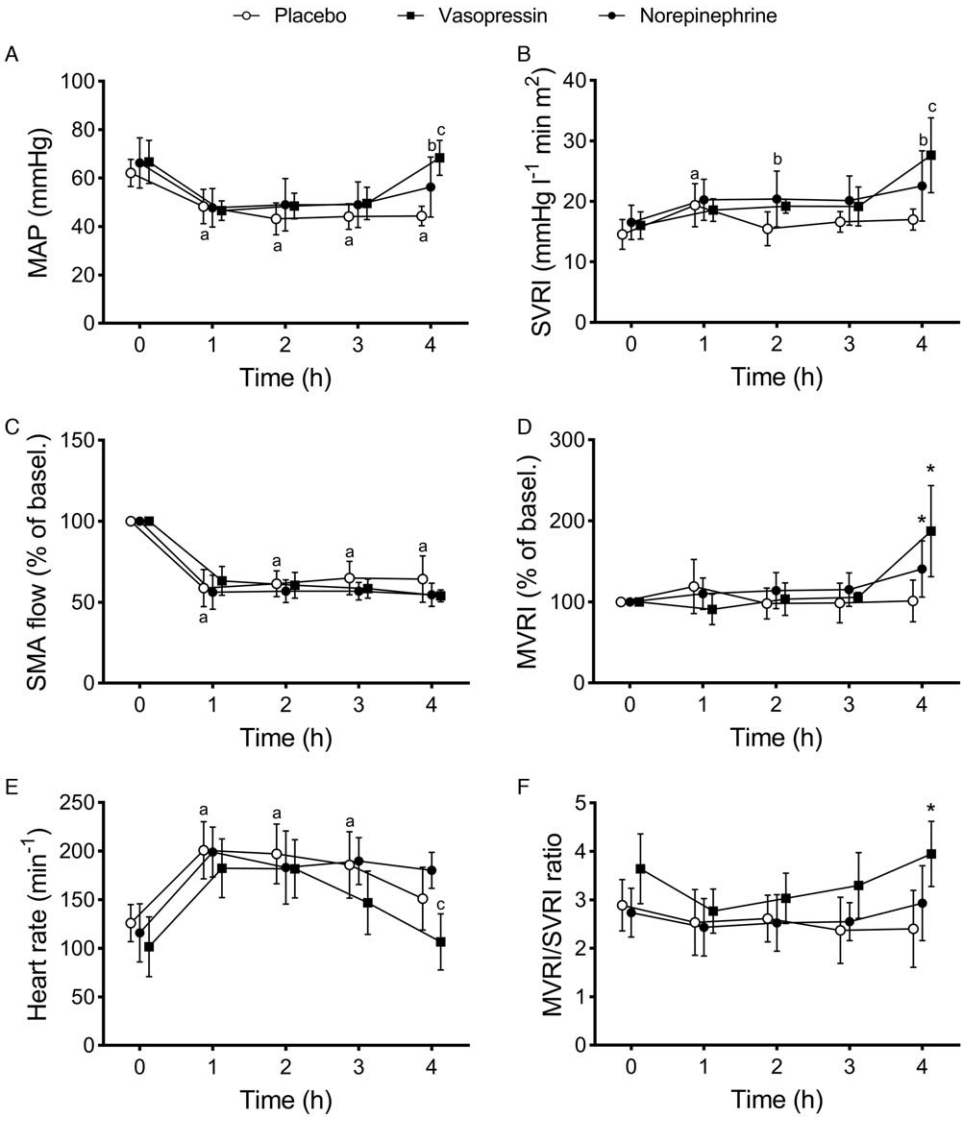

Fig. 4. **Mean arterial pressure (MAP, panel A), mesenteric and systemic vascular resistance indices, and their ratio (MVRI, panel D; SVRI, panel B; MVRI/SVRI ratio, panel F), superior mesenteric artery blood flow (SMA flow, panel C), and heart rate (panel E) in animals subjected to partial inflation of an occlusion balloon in the inferior caval vein immediately after time point 0 h.** Starting at 1 h, the animals were randomized to intravenous infusion of either placebo (5% glucose, 0.2 mL kg^{-1} h^{-1}), vasopressin (0.001 U kg^{-1} min^{-1} for the first hour, 0.002 U kg^{-1} min^{-1} for the second hour, and 0.006 U kg^{-1} min^{-1} for the last hour) or norepinephrine (0.04 μg kg^{-1} min^{-1} for the first hour, 0.12 μg kg^{-1} min^{-1} for the second hour, and 0.36 μg kg^{-1} min^{-1} for the last hour). [a]: statistical difference between the indicated time point and baseline in the placebo group. [b] and [c]: statistical difference between the norepinephrine or vasopressin and placebo group, respectively, at the indicated time points. Data are presented as means and 95% confidence intervals. See Figure 1 for the exact number of animals in each group at each time point.

DISCUSSION

The main finding of this study was that the inodilators, levosimendan, and milrinone have negligible vasodilatory effects on the gastrointestinal circulation in fixed low CO, thus rejecting the original hypothesis of this study. Another finding was that the vasopressors, vasopressin, and norepinephrine have potent dose-dependent vasoconstrictor effects in the systemic and mesenteric vascular beds.

The experimental model

In this study, inflation of an inferior caval vein balloon was used to simulate acute, severe, cardiac dysfunction through a predominant decrease in preload. This model does not simulate cardiac dysfunction from the perspective of the heart but elicits many of the intestinal consequences of this state, including low CO and MAP, reduced blood flow in the SMA, as well as increased MVP indicating mesenteric venous stasis (14). This model is therefore reasonable for investigations of drug effects on the gastrointestinal tract. Other relevant models include induction of cardiac tamponade, direct aortic, or mesenteric in-flow restriction and open- and closed-chest models of acute cardiac ischemia (15, 22–26). However, in this study, the intention was to induce and maintain a predetermined level of CO, which was accomplished, to isolate peripheral vasoactive effects from inotropy. Based on our previous work using a protocol with down titration of CO, the level of reduction was set to 60% of the baseline value (14). Cardiac output was monitored by a semi-continuous method, and the basic physiology of the animals, e.g., oxygenation, body temperature, and fluid balance, was carefully kept constant in all groups. Furthermore, randomization was employed to reduce selection bias.

Effects of low cardiac output

The lowered CO led to a decrease in MAP and a compensatory increase in heart rate. SMA blood flow decreased in parallel with the decreased CO. The model implicates an artificial resistance in the inferior caval vein, which may complicate the calculation of SVR, in particular where outflow pressure should be measured. Central venous pressure was measured proximal to the balloon to correspond to the measured flow in the calculation, i.e., CO, thus the calculated SVR includes both the endogenous resistance and the resistance of the balloon, which may obscure the interpretation of the biological SVR produced by the study drugs. However, due to the small pressure gradient between MVP and CVP, we believe that the resistance must be low in relation to the resistance produced by the arterioles. Furthermore, the resistance of the balloon is inert to the study drugs and the inflated volume was almost constant throughout the experiments. Lactate concentrations increased and arterial pH decreased due to the hypoperfusion and inadequate oxygen delivery. Other confounding factors, such as hypoxemia or anemia, were eliminated. From the present data, the exact source of the lactate increase caused by low CO cannot be identified. Arguably, part of the lactate originates from the intestines due to numerically higher intraperitoneal than arterial lactate concentrations, and a tendency for a negative arterial to venous mesenteric lactate difference. Notably, the metabolic response to low CO seems to be adaptive, i.e., the metabolic changes returned toward normal over time. This dynamic makes the comparison with the drug intervention groups troublesome; however, also showing the importance of a placebo group over time in this study.

The inodilators

Levosimendan is believed to be a calcium sensitizer, but it has been suggested that the main mechanism is phosphodiesterase III inhibition (27, 28). Milrinone is only a phosphodiesterase III-inhibitor (8). In this study, these two inotropic drugs did not show any significant effects on the mesenteric circulation, which may be due to the presumptive endogenous vasoconstriction induced by the low CO. However, levosimendan increased lactate concentrations in arterial, mesenteric venous, and mixed venous blood samples. These elevations in lactate concentrations indicate an increased systemic anaerobic metabolism, possibly due to hypoperfusion or other metabolic effects caused by levosimendan since other confounding factors were eliminated. Milrinone did not affect the metabolism significantly. Both levosimendan and milrinone increased the intraperitoneal glycerol concentration, which may be due to cell damage (29) or enhanced lipolysis by phosphodiesterase III inhibition (30). However, it must be noted that the analysis at the latest time point comprised a low number of animals. Unexpectedly, there were no differences in the balloon deflation volume over time between the groups. In theory, it could have been necessary to increase the inflated balloon volume due to improved contractility by the inodilators. However, one plausible explanation to the contrary finding may be the provoked systemic hemodynamic and metabolic disturbances causing myocardial depression.

The present findings on levosimendan in a model with fixed CO are partly in conflict with previous research in naive animals and patients in whom CO was able to increase. In unmanipulated dogs, levosimendan increased the blood flow to the small intestines and decreased the splanchnic vascular resistance (10), and was superior to milrinone in increasing gastric mucosal oxygenation (11). Levosimendan increased gastric mucosal perfusion in a study on septic shock patients with left ventricular dysfunction (31). In open abdominal aortic aneurysm surgery, levosimendan increased gastric mucosal perfusion but did not affect total splanchnic blood flow (32). In low CO states after cardiac surgery, levosimendan increased hepatic blood flow in relation to the increase in CO and reduced the resistance in the hepatic artery (33).

Previous research on milrinone is not conclusive regarding splanchnic effects. Milrinone did not alter the splanchnic vascular resistance when given to unmanipulated dogs (10). In a placebo-controlled randomized trial on 22 patients undergoing coronary artery bypass grafting, milrinone infusion resulted in reduced gastric mucosal acidosis 24 h after surgery as well as lower levels of inflammatory markers and endotoxin (34). Milrinone, dopamine, and placebo were compared during coronary artery bypass grafting and no differences in release of endotoxins or gastric mucosal pH were found intraoperatively (35).

Importantly, our results do not imply that milrinone and levosimendan are ineffective in clinical cardiac dysfunction by inotropy where an increase in CO is permitted, since sufficient forward flow is assumed to increase mesenteric perfusion. The findings in this study do, however, argue against that there is a pharmacological vasodilation *per se* in low CO. This observation is applicable in all condition with low CO when the inodilators are used and especially if splanchnic circulatory compromise is clinically suspected.

The vasopressors

In the present study, norepinephrine and vasopressin increased both the systemic and the mesenteric vascular resistances in low CO. Additionally, vasopressin increased the MVRI/SVRI ratio, indicating that vasopressin had a greater constricting effect on the mesenteric circulation. Despite the fact that vasopressin increased MAP, vasopressin did not change SMA blood flow significantly and neither did norepinephrine. A plausible explanation could be that CO was kept constant in this model and that the proportion of SMA flow to CO was almost fixed. The blood flow to the various layers in the gastrointestinal tract is autoregulated (36), which may diminish the circulatory and metabolic responses seen in this model. Also, reduced organ perfusion in this model probably caused an endogenous compensatory vasoconstrictive response, potentially interacting with the circulatory effects of both vasopressors. This may explain that a high dose of the vasopressors was needed for a vasoconstrictive effect in this model. However, the importance of increased mesenteric resistance should not be underestimated since it might lead to localized severe hypoperfusion in parts of the intestines and hypoxia in peripheral intestinal epithelial cells. One indication of these potential processes was that the intraperitoneal lactate concentration and lactate/pyruvate ratio increased at the highest vasopressin dose. Notably, there was no statistical significant change in mesenteric oxygen consumption or arterial and mesenteric venous lactate concentrations. This could be due to that a localized intestinal ischemia may not be large enough to be observed in a gross calculation of oxygen consumption or blood lactate but still be detectable intraperitoneally (37, 38).

These results complement previous research in other shock models and in humans, which have also indicated that vasopressin provokes stronger vasoconstriction in the splanchnic circulation than norepinephrine. In a porcine sepsis model, infusion of vasopressin increased MAP but reduced mesenteric blood flow and small intestinal perfusion, while colonic perfusion was unchanged (39). Vasopressin used to preserve MAP after transient myocardial ischemia in pigs reduced CO and blood flow to the heart, brain, and kidneys (40). During extracorporeal circulation in pigs, rectosigmoidal mucosal perfusion was maintained but mucosal ischemia developed after vasopressin was given (41). Vasopressin was found to cause intestinal and gastric mucosal vasoconstriction in patients in norepinephrine-treated vasodilatory shock after cardiac surgery when MAP was kept at 75 mm Hg (42). Also, in the context of cardiac surgery, endogenous vasopressin has been suggested to produce vasospasm in vascular grafts during coronary artery bypass grafting (43).

In a porcine model of sepsis, norepinephrine increased perfusion pressure and CO while flow in the SMA and mucosal perfusion declined (44). However, some previous studies have suggested that norepinephrine may be safe regarding splanchnic circulation. In an endotoxemic shock study in sheep, when MAP was restored using norepinephrine, SMA blood flow and mucosal perfusion were unaltered (45). Norepinephrine titrated to MAP 60 mm Hg to 65 mm Hg or 80 mm Hg to 85 mm Hg during extracorporeal circulation showed no difference in splanchnic effects (46). It must be acknowledged that the highest dose of vasopressin used in this study is higher than that normally used in a clinical setting, and that this reasoning is not supported by histological analysis, which would have strengthened the study. However, this study in fixed low CO together with previous experimental studies in other shock models and human research put attention to that the vasopressors has dose-dependent unwanted vasoconstrictive effects in the splanchnic circulation. Norepinephrine has dose-dependent inotropic effects by beta-adrenergic, and possibly alpha-adrenergic, agonism, and may be better tolerated if CO was allowed to increase (8, 47).

Limitations

First, the study was performed in an animal model; therefore, the results may not be directly transferable to humans. Second, the number of animals in the levosimendan and milrinone groups was low at the last time point due to loss of animals, and several groups were compared, which might explain why the statistical analyses showed no significant differences, although there were apparent numerical differences in some variables. Third, the present study does not investigate a combination of drugs, the use of which is common in intensive care. However, this is also a strength since it was possible to explore the effects of each drug when used alone in low CO. To advance clinical knowledge, a future study could include an investigation of inodilators in combination with vasopressors. Fourth, as a marker of severe deterioration with presumed hepatocyte damage by the suprahepatic placement of the balloon, plasma glucose concentrations became very low in some animals. To compensate for this limitation, animals with very low MAP and/or plasma glucose concentration were excluded. Fifth, the present study aimed at elucidating the magnitude of direct vasoactivity of the investigated drugs at fixed CO, limiting the translation to the entire clinical situation. However, the aim of the present study was to explore the pharmacological effect on splanchnic vessel resistance independent of inotropic influences of the drugs. Probably, improved splanchnic perfusion is best achieved by improving CO.

CONCLUSION

Levosimendan and milrinone had negligible vasodilatory effects on the gastrointestinal circulation in fixed low CO, showing that the direct, perhaps beneficial, vasodilator effects of these inodilators may be absent in this condition. This finding rejects the original hypothesis and questions splanchnic vasodilation as a valid argument *per se* for clinical use of these

drugs. Vasopressin and norepinephrine at high doses caused vasoconstriction in the gastrointestinal circulation and vasopressin affected the mesenteric circulation more than the systemic, which may have anaerobically manipulated the intraperitoneal metabolism, suggesting potential side effects.

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