

Shunting of the Microcirculation After Mesenteric Ischemia and Reperfusion Is a Function of Ischemia Time and Increases Mortality

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ABSTRACT

Objective: Shunting of the microcirculation contributes to the pathology of sepsis and septic shock. The authors address the hypothesis that shunting of the microcirculation occurs after superior mesenteric artery occlusion (SMAO) and reperfusion, and explore functional consequences.

Methods: Spontaneously breathing animals (rats) ($n = 30$) underwent SMAO for 0 (controls), 30 (SMAO₃₀) or 60 min (SMAO₆₀) followed by reperfusion (4 h) with normal saline. Leukocyte–endothelial interactions in mesenteric venules were quantified in an exteriorized ileal loop using intravital microscopy. Abdominal blood flow was recorded continuously, and arterial blood gases were analyzed at intervals. The above groups were matched by comparable groups with continuous superior mesenteric artery blood flow measurements and without exteriorizing an ileal loop (controls*, SMAO₃₀*, SMAO₆₀*).

Results: Adherent leukocytes increased shortly after reperfusion in ischemia groups, and plateaued in these groups. Centerline velocity in the recorded venules was significantly reduced after reperfusion down to low-flow/no-flow in SMAO₆₀ as compared to SMAO₃₀ and controls, whereas perfusion of the SMA and ileal vessels persisted. The microcirculatory changes in SMAO₆₀ were accompanied by progressive metabolic acidosis, substantially larger volumes of intravenous fluids needed to support arterial blood pressure and significantly reduced survival (30%). SMA blood flow increased in relation to abdominal blood flow after reperfusion in SMAO₆₀*, and remained constant in SMAO₃₀* and controls*. Survival was 80% in SMAO₆₀*.

Conclusion: Shunting of the microcirculation can be observed after SMAO for 60 min and reperfusion, and contributes significantly to the pathology of mesenteric ischemia and poor outcome.

Microcirculation (2006) **13**, 411–422. doi:10.1080/10739680600746032

KEY WORDS: ischemia, mesentery microcirculation, mortality, reperfusion, shunting

Perfusion heterogeneities in micro- and macrocirculation, such as shunting of the microcirculation, are usually described in the context of sepsis and septic shock (21). Depending on the metabolic demand of

individual tissues in which perfusion heterogeneities occur, this may have no effect at all or alter tissue function and aggravate tissue damage (23). However, little is known about the impact of these perfusion abnormalities on patient outcome and survival (24). Furthermore, treatment options and methods to visualize the effect of perfusion abnormalities on key microcirculatory regions like the mesentery are still limited (23,24). As mesenteric arteries nourish a highly active tissue with high cellular turnover and a barrier function that is essential for the organism, a perfusion mismatch most likely endangers the function and integrity of this tissue (6).

As previously reported, perfusion abnormalities reproducibly occurred in systemic and regional macrocirculation together with changes in systemic

We are grateful to Mr. Kopez, A. Heimann and Mr. Malzahn for their excellent assistance. This work was supported by the Else Kröner-Fresenius-Stiftung and the University of Mainz (MAIFOR). The manuscript includes data from the doctoral thesis of Nicola Plum.

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Received 17 May 2005; accepted 12 February 2006.

and regional vascular resistance after 60 min of superior mesenteric artery ischemia and 4 h of reperfusion (14). The current study was designed to evaluate the effect of superior mesenteric artery ischemia and reperfusion on microcirculatory perfusion and leukocyte/endothelial interaction in the SMA-dependent microvascular bed using intravital microscopy, and correlate this with the macrocirculatory perfusion abnormalities seen in previous experiments (14). We raise the hypothesis that these changes are caused by shunting of the microcirculation. For this, we used intravital microscopy in an improved animal model with continuous hemodynamic monitoring, intermittent testing of blood gases, and analysis of selected metabolic parameters (9,14).

MATERIALS AND METHODS

Thirty male Sprague-Dawley rats (body wt 350 ± 10 g) were maintained on a standard rat chow and water ad libitum before the experiment. After anesthesia with urethane (1.25 g/kg body wt IM, single dose), the carotid artery and jugular vein were cannulated with a small PE-tube for arterial blood pressure recordings, arterial blood gas analyses, and fluid replacement during the experiment via central venous line. For each sample 250 μ L of blood was drawn into heparinized syringes. Arterial blood gases p_aO_2 , pH, cBase(ecf) (BE), hematocrit (Hct), as well as lactate, potassium, sodium, and chloride, were analyzed with Arterial Blood Gas Laboratory Radiometer Copenhagen 615. p_aO_2 , p_aCO_2 , and arterial pH, hemoglobin concentration, lactate, potassium, and sodium were measured, whereas BE and Hct were calculated from measured values (13).

Rats received a basal infusion with albumin in physiological saline (0.3 mL/100 g body wt/h) for compensation of intraoperative albumin loss and evaporative water loss as previously published (10). The animals were placed on a heating pad, and the rectal temperature was kept constant at $37.5 \pm 0.5^\circ\text{C}$ by means of a feedback-controlled heating unit (Homeothermic Blanket Control, Harvard, South Natick, MA, USA). After a median laparotomy, a Doppler flow probe (ES-20-1.6, Triton Technology, San Diego, CA, USA) was placed around the abdominal aorta without impairing the normal blood flow in this vessel. A snare with a monofilament suture was placed around the superior mesenteric artery (SMA) serving as occlusion apparatus during the ischemic period as previously described (14). Before an ileal loop was exteriorized for intravital microscopy (see

below), a second Doppler flow probe (ES-20-1.0, Triton Technology) was placed around the origin of the SMA to obtain baseline Doppler flow readings of this vessel. This second flow probe was removed while an ileal loop was exteriorized for intravital microscopy in order not to impair the perfusion of the SMA. At the end of the experiments, the Doppler flow probe was re-positioned around the SMA.

Hemodynamic data (systolic, mean and diastolic arterial blood pressure [SAP, MAP, DAP], heart rate [HR], abdominal blood flow) were recorded using System 6 (Triton Technology), digitized and registered on a beat-to-beat basis with a computer-based system (Dasylab, National Instruments Corporation, Austin, TX, USA). Abdominal stroke volume was calculated real-time from the area under the curve of the registered pulsatile velocity curves. Abdominal blood flow (ABF) was calculated by multiplication of the abdominal stroke volume and the simultaneously registered heart rate. Superior mesenteric artery blood flow was calculated correspondingly. The Doppler flow values showed a linear correlation with electromagnetic blood flow sensors (Skalar-Medical, Delft, The Netherlands) as previously described (8). An ECG in lead II was recorded during the entire duration of the experiment to detect ECG-changes of any sort.

Animals were placed with the heating pad underneath in the left lateral recumbent position on an adjustable Plexiglas microscope stage. A segment of the ileum was exteriorized through the abdominal incision avoiding trauma to the exposed bowel and mesentery. The ileal loop was covered with oxygen-impermeable plastic foil (Folio, Germany) to prevent evaporative loss of water (8). The exposed bowel segment was continuously superfused with warm (37.5°C) Krebs-Henseleit-buffer, the pH of the buffer was adjusted to pH 7.4 with 5% CO_2 in N_2 .

Intravital microscopy was performed with epi- and transillumination observing 3–5 unbranched mesenteric venules (diameter 20–35 μm ; length 125–180 μm) (Zeiss Axiotech fluorescence microscope with computer-controlled scanning table; light source: AttoArc HBO 100 W). The images were recorded with a high-resolution camera (Stemmer b/w VS 450) and a videocassette recorder (S-VHS Panasonic AG-7355). Off-line analysis was performed with the Cap Image software system (Dr. Zeintl, Heidelberg, Germany, Version 6.01) on an IBM-compatible PC with a Matrox image processing card and real-time video tape digitization.

Erythrocyte centerline velocity was measured with a frame-to-frame method using fluorescein isothiocyanate (FITC)-labeled red blood cells from donor animals (8,9). Wall shear rate (γ) was calculated based on the Newtonian definition: $\gamma = 8 * (\text{mean velocity}/\text{venular diameter})$; mean velocity was calculated by dividing maximum velocity by an empirical factor of 1.6 as previously described (8).

Rolling and adherent leukocytes were analyzed at distinct time points (see experimental protocol) using a frame-to-frame method for an observation period of 1 min each for epi- and transillumination as previously published (8). A leukocyte was defined as adherent to venular endothelium if it remained stationary for >30 s under flow. Rolling and adherent leukocytes were expressed as number per millimeter squared calculated from the measured diameter and length of the observed vessels assuming cylindrical geometry.

For continuous superior mesenteric artery flow measurements, a second set of experiments were performed using the same setup without exteriorizing an ileal loop for intravital microscopy. The two Doppler flow probes remained in position throughout the whole observation period, and the abdominal wound was sealed off around the flow probe wires with oxygen-impermeable plastic foil (Folio, Germany). For this second set of experiments, 30 male Sprague-Dawley rats of comparable weight and age were used.

All investigative procedures and the animal facilities conformed with the *Guide of Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health. The regional animal care and use committee approved the protocol.

Experimental Protocol

Animals were randomized into 6 groups (10 animals per group): In SMAO_30 the SMA was occluded by tightening the snare for 30 min, in SMAO_60 the SMA was occluded for 60 min. Animals, who underwent the same operative procedure without tightening of the snare served as controls. Follow-up of SMAO_30 and SMAO_60 was carried out for 4 h after release of the snare. Sham were followed up for a total of 5 h. Each of these 3 groups was matched by a group that did not undergo exteriorization of an ileal loop for intravital microscopy (controls*, SMAO_30*, SMAO_60*).

Mean arterial blood pressure was kept above 70 mmHg by infusion of normal saline (0.9%) by cen-

tral venous line infusion (8,10). At baseline (prior to the occlusion) of the SMA, at the beginning of reperfusion, and at 30, 60, 120, 180, 240 min of reperfusion (R, 30R, 60R, 120R, 180R, and 240R) 3 to 5 unbranched mesenteric venules were studied by intravital microscopy (only controls, SMAO_30, SMAO_60), and arterial blood gases (ABG) were drawn in all groups (controls, SMAO_30, SMAO_60, controls*, SMAO_30*, SMAO_60*). Rhodamine-6-G (100 μ L of a 0.005% solution, Sigma Aldrich) was injected via the central line to visualize leukocytes using epi-illumination at each designated recording time point. Red blood cell centerline velocity was recorded using FITC-labeled red blood cells from donor animals as previously described (9). After termination of the experiments, the SMA was dissected and morphologically analyzed to ensure the integrity of the vessel at the former position of the occluding snare (14).

Statistical Analysis

Data are presented as means \pm SEM. Statistical analysis was performed with Sigma Stat (SPSS Science, Chicago, IL). In animals subjected to exteriorization of an ileal loop (controls, SMAO_30, SMAO_60) until 120 min after reperfusion (120R), and in animals not subjected to intravital microscopy (controls*, SMAO_30*, SMAO_60*) at all time points, statistical significance of changes from baseline values within each group, and differences between groups were tested with two-way analysis of variance (ANOVA) for repeated measures (one factor repetition: "within-subjects"). A multiple comparison procedure was performed using the Holm-Sidak method. Statistical significance was accepted at $p < .05$. With the high death rate after 120R in animals subjected to 60 min of mesenteric ischemia and exteriorization of an ileal loop, data from this group (SMAO_60) were analyzed descriptively only at time points after 120R. In the two other groups with exteriorization of an ileal loop (SMAO_30, controls), differences between animals were tested with a Student t test at time points after 120R. To exclude the possibility that differences at baseline were carried forward, values at time points 180R and 240R were subtracted from baseline values of these groups and the results compared with a Student t test. Likewise, statistical significance was accepted at $p < .05$.

RESULTS

Sham group animals (controls, controls*) remained at baseline levels in terms of all parameters measured

throughout the observation period except for a slight increase in heart rates over the course of the experiment. To avoid presenting redundant data, mainly data from groups with exteriorization of an ileal loop for intravital microscopy (controls, SMAO_30, SMAO_60) are given. And only important differences between these and groups without exteriorization of an ileal loop and continuous superior mesenteric artery blood flow measurements (controls*, SMAO_30*, SMAO_60*) are given.

Outcome

All sham-operated animals and all animals undergoing 30 min of ischemia and subsequent reperfusion (SMAO_30) survived until the end of the observation period. The survival rate was significantly lower (30%) in SMAO_60 (60 minutes of ischemia) as compared to SMAO_30 (100%) and controls (100%). The Kaplan-Meier survival analysis log-rank (Figure 1) illustrates the temporal dying pattern. Data from animals that did not survive the entire course of the experiment were included in the data to the point of their death.

Volume Substitution

Sodium chloride 0.9% was infused to maintain mean arterial blood pressure above 70 mmHg. Controls did not require any volume in addition to basal infusion. The volume needed to support blood pressure in SMAO_60 (66.0 ± 18.0 mL) was significantly larger

as compared to SMAO_30 (8.8 ± 3.0 mL) and controls ($p < .05$); so was the volume in SMAO_30 as compared to controls ($p < .05$).

Mesenteric Microcirculation

Rolling and Adherent Leukocytes. The number of rolling and adherent leukocytes was counted only in perfused postcapillary venules as no-flow completely prevented recruitment of leukocytes to the areas of interest. At baseline, the number of rolling leukocytes was $176 \pm 19/\text{mm}^2$ in controls, $187 \pm 29/\text{mm}^2$ in SMAO_30 and $122 \pm 24/\text{mm}^2$ in SMAO_60. Except for a transient, significant increase in the number of rolling leukocytes, which occurred in SMAO_30 and SMAO_60 shortly after reperfusion (controls: $187 \pm 21/\text{mm}^2$, SMAO_30: $367 \pm 63/\text{mm}^2$, SMAO_60: $431 \pm 55/\text{mm}^2$, $p < .05$), there was no difference in the number of rolling leukocytes between the groups during the experiments. Adherent leukocytes are depicted in Figure 2. In ischemia groups, the number of adherent leukocytes increased significantly shortly after reperfusion ($p < .05$). In SMAO_30, the number of adherent leukocytes plateaued after 60 min of reperfusion until the end of observation, whereas in SMAO_60 the number of adherent leukocytes tended to decrease until 60R (60 min after reperfusion). Thereafter, adherent cells increased significantly ($p < .05$) in SMAO_60 to similar levels as in SMAO_30 until 120R. From the 3 surviving animals, only one animal had persistent microvascular perfusion. In this animal the number of adherent leukocytes increased further until the end of the experiments (Figure 2, black squares).

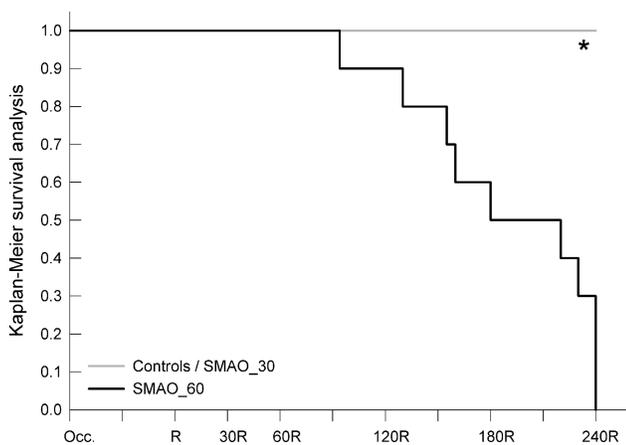


Figure 1. Kaplan-Meier survival analysis log-rank. SMAO_30: ischemia duration 30 min, SMAO_60: ischemia duration 60 min; Occ.: superior mesenteric artery occlusion; R, 30R, 60R, 120R, 180R, 240R: reperfusion, numbers indicate minutes. *Significant difference between SMAO_60 and controls, SMAO_30, $p < .05$.

Centerline Velocity and Shear Rate. Centerline (red blood cell) velocity in the recorded mesenteric venules was significantly lower in ischemia groups as compared to control animals after reperfusion until 120R (Figure 3a), the decrease in centerline velocity being inversely correlated with ischemia time. After 120R, centerline velocity remained significantly lower in SMAO_30 compared to controls, and continued to be lower in SMAO_60 in surviving animals until the end of the experiments. The mean vessel diameters are displayed in Figure 3a. There were no significant differences between the groups and changes from baseline in each group were not significant. Shear rate data paralleled centerline velocity data during the course of the observation (Figure 3b). Perfusion of ileal arteries returned after release of the occluding snare and persisted until the end of observation. The number of adherent cells increased in the ileal

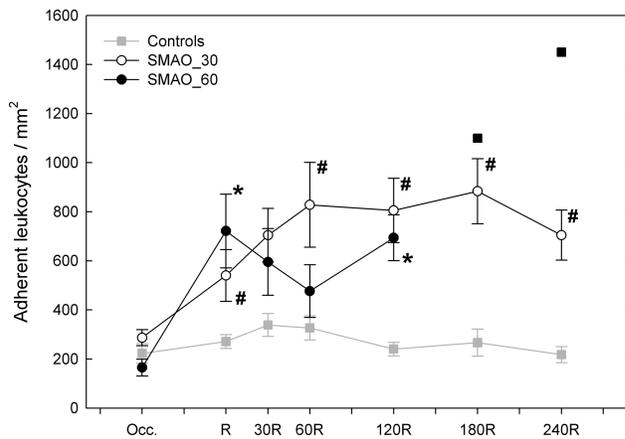


Figure 2. Adherent leukocytes/mm². Data are presented as means ± SEM. SMAO_30: ischemia duration 30 min, SMAO_60: ischemia duration 60 min; Occ.: superior mesenteric artery occlusion; R, 30R, 60R, 120R, 180R, 240R: reperfusion (numbers indicate minutes). #Significant difference between SMAO_30 and controls, $p < .05$; *significant difference between SMAO_60 and controls, $p < .05$; ~significant difference between SMAO_30 and SMAO_60, $p < .05$. Black squares: adherent leukocytes/mm² in surviving animal from SMAO_60 with persistent microvascular perfusion.

venules, but could not be quantified using our experimental setting.

Hemodynamics

Mean Arterial Blood Pressure (MAP), Heart Rates (HR). MAP did not differ between groups at the beginning of experiments. As depicted in Figure 4, MAP decreased in SMAO_60 shortly after reopening of the SMA and remained significantly lower from 60R until 120R as compared to controls ($p < .05$). In surviving animals, MAP did not increase in SMAO_60 until 240R. In SMAO_30, MAP fell less steeply compared to SMAO_60 and was significantly lower compared to control animals from 180R to 240R ($p < .05$). There was no significant difference in MAP between both ischemia groups at any time point during the experiments. Heart rates (Figure 5) increased in SMAO_60 from the end of the ischemic period. From 60R until 120R, heart rates were significantly higher in SMAO_60 as compared to controls ($p < .05$), and heart rates increased further in surviving animals until the end of the experiments. In SMAO_30, heart rates increased nonsignificantly from 60R until 240R.

Abdominal Blood Flow (ABF). ABF continuously decreased in SMAO_30 until 240R with significant

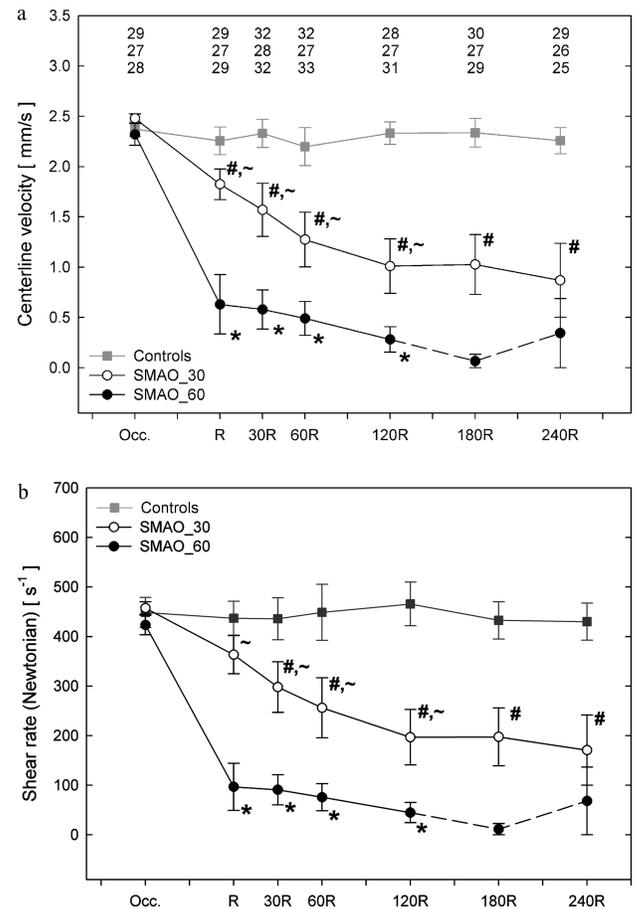


Figure 3. Centerline velocity, shear rate, Data are presented as means ± SEM SMAO_30: ischemia duration 30 min, SMAO_60: ischemia duration 60 min; Occ.: superior mesenteric artery occlusion; R, 30R, 60R, 120R, 180R, 240R: reperfusion (Numbers indicate minutes). #Significant difference between SMAO_30 and controls, $p < .05$; *significant difference between SMAO_60 and controls, $p < .05$; ~significant difference between SMAO_30 and SMAO_60, $p < .05$. Data from SMAO_60 at 180R and 240R were not compared to the other groups with a statistical test due to a significantly reduced group size. These time points are connected to the previous time point with a dashed line. Numbers in the figure: Mean venular diameter, 1st row controls, 2nd row: SMAO_30, 3rd row: SMAO_60.

($p < .05$) differences (as compared to controls) from 120R until the end of the experiments (Figure 6). In SMAO_60, ABF decreased significantly during ischemia and until 60R as compared to SMAO_30 and controls ($p < .05$). Thereafter until 120R, ABF increased in SMAO_60 in surviving animals to comparable values as in SMAO_30. The inset graph in Figure 6 shows ABF only in animals from SMAO_60 that survived until 240R. Typically, only surviving

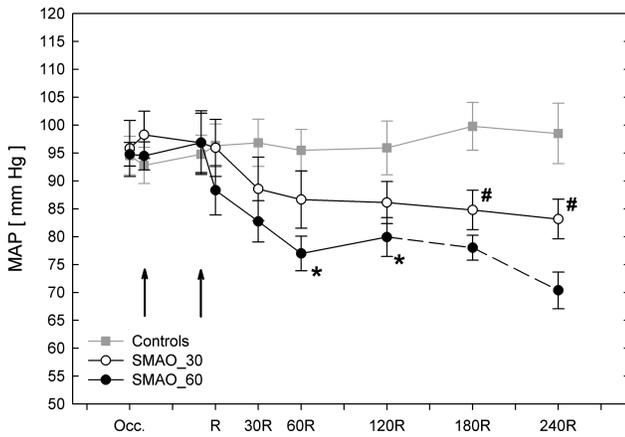


Figure 4. Mean arterial blood pressure (MAP). Data are presented as means \pm SEM. SMAO_30: ischemia duration 30 min, SMAO_60: ischemia duration 60 min; Occ.: superior mesenteric artery occlusion; R, 30R, 60R, 120R, 180R, 240R: reperfusion (numbers indicate minutes). First arrow: Occ. + 10 min, second arrow: 10 min before R. #Significant difference between SMAO_30 and controls, $p < .05$; *significant difference between SMAO_60 and controls, $p < .05$. Data from SMAO_60 at 180R and 240R were not compared to the other groups with a statistical test due to a significantly reduced group size. These time points are connected to the previous time point with a dashed line.

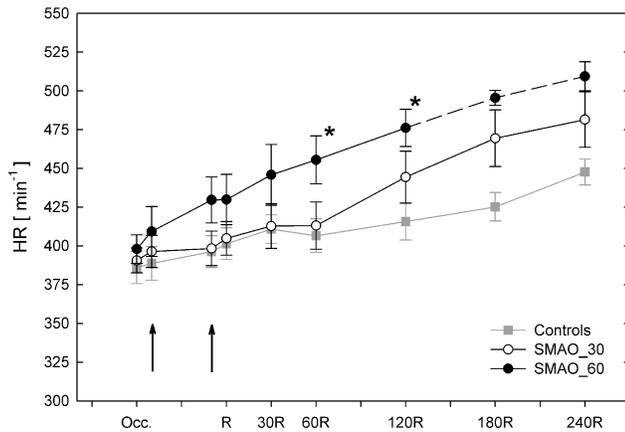


Figure 5. Heart rate (HR). Data are presented as means \pm SEM. SMAO_30: ischemia duration 30 min, SMAO_60: ischemia duration 60 min; Occ.: superior mesenteric artery occlusion; R, 30R, 60R, 120R, 180R, 240R: reperfusion (numbers indicate minutes). First arrow: Occ. + 10 min, second arrow: 10 min before R. *significant difference between SMAO_60 and controls, $p < .05$. Data from SMAO_60 at 180R and 240R were not compared to the other groups with a statistical test due to a significantly reduced group size. These time points are connected to the previous time point with a dashed line.

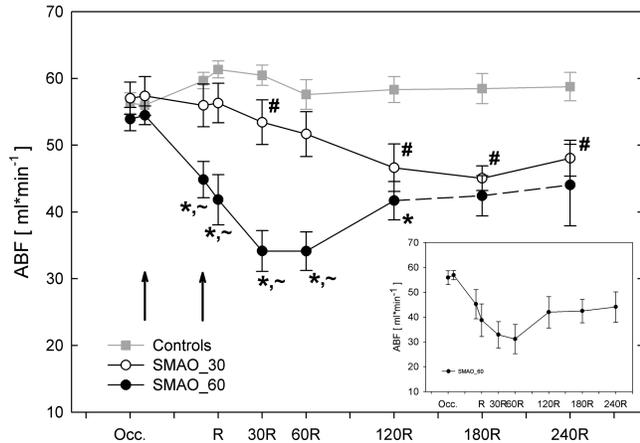


Figure 6. Abdominal blood flow (ABF). Data are presented as means \pm SEM. SMAO_30: ischemia duration 30 min, SMAO_60: ischemia duration 60 min; Occ.: superior mesenteric artery occlusion; R, 30R, 60R, 120R, 180R, 240R: reperfusion (numbers indicate minutes). First arrow: Occ. + 10 min, second arrow: 10 min before R. #Significant difference between SMAO_30 and controls, $p < .05$; *significant difference between SMAO_60 and controls, $p < .05$; ~significant difference between SMAO_30 and SMAO_60, $p < .05$. Inset graph shows only ABF in animals from SMAO_60 that survived until 240R. Data from SMAO_60 at 180R and 240R were not compared to the other groups with a statistical test due to a significantly reduced group size. These time points are connected to the previous time point with a dashed line.

animals showed recovery of ABF toward the end of the experiments.

Superior Mesenteric Artery Blood Flow (MBF). MBF was obtained before baseline and after the final intravital microscopy tape recordings (Table 1). MBF was comparable in all groups before baseline, increased significantly in controls ($p < .05$), and tended to increase in SMAO_60, whereas MBF decreased significantly in SMAO_30 from baseline until after 240R. MBF was significantly lower in SMAO_30 compared to control animals after 240R ($p < .05$). In surviving animals in SMAO_60, MBF was comparable to controls.

Table 1. Superior mesenteric artery blood flow (MBF) at baseline and 240R

	Baseline	240R
MBF (mL*min ⁻¹)		
Controls	12.1 \pm 1.4	14.9 \pm 0.9 ⁺
SMAO_30	11.5 \pm 0.6	8.8 \pm 1.0 ^{+,#}
SMAO_60	11.7 \pm 0.9	13.5 \pm 2.3

⁺Significant difference between 0 and 240R, $p < .05$.

[#]Significant difference between SMAO_30 and controls, $p < .05$.

Continuous Superior Mesenteric Artery Blood Flow Measurements (cMBF), cMBF as Percentage of ABF. *For these experiments, the setup was modified in that the superior mesenteric artery flow probe remained in place during the entire experiment and an ileal loop was not exteriorized. All animals survived in the SMAO_30* group and controls, and 80% of the animals survived in the SMAO_60* group until the end of the experiments. Although the changes in blood gas parameters in response to ischemia were less pronounced in these animals compared to animals with exteriorization of an ileal loop, the pattern of changes was identical.

Superior mesenteric artery blood flow was comparable at baseline (Figure 7a). After release of the occluding snare, MBF was lower in animals subjected to 30 and 60 min of ischemia compared to controls. After 120R, MBF increased in SMAO_60* to levels as found in controls, whereas MBF remained lower in SMAO_30* until the end of the experiments. MBF remained constant in relation to ABF in SMAO_30*, whereas MBF increased continuously in SMAO_60* after release of the occluding snare with significant differences between SMAO_30* and SMAO_60* from 180R to 240R (Figure 7b).

Arterial Blood Gas Values

Blood pH did not differ between the groups at the beginning of the experiments (Table 2). In SMAO_30, blood pH tended to decrease until 30R. However, over the course of the observation period there was no significant difference between SMAO_30 and controls, whereas in SMAO_60 blood pH was significantly lower than in controls from 60R until 120R ($p < .05$), and significantly lower ($p < .05$) compared to SMAO_30 at 120R (Table 2). Blood pH decreased further in surviving animals in SMAO_60. p_aCO_2 did not differ between all groups at the beginning of the experiments (Table 2) or at R. p_aCO_2 decreased thereafter in SMAO_30 and SMAO_60 until the end of the experiments, the decrease being significant ($p < .05$) in SMAO_30 as compared to controls from 120R to 180R (Table 2). Without statistical significance, p_aCO_2 tended to be lower in SMAO_60 compared to controls and SMAO_30 at 120R, and in surviving animals at 240R. Corresponding base excess values are depicted in Figure 8. Base excess values decreased significantly in both ischemia groups versus control from 30R ($p < .05$). Both ischemia groups differed significantly in base excess with lower values in SMAO_60 from 30R until 120R

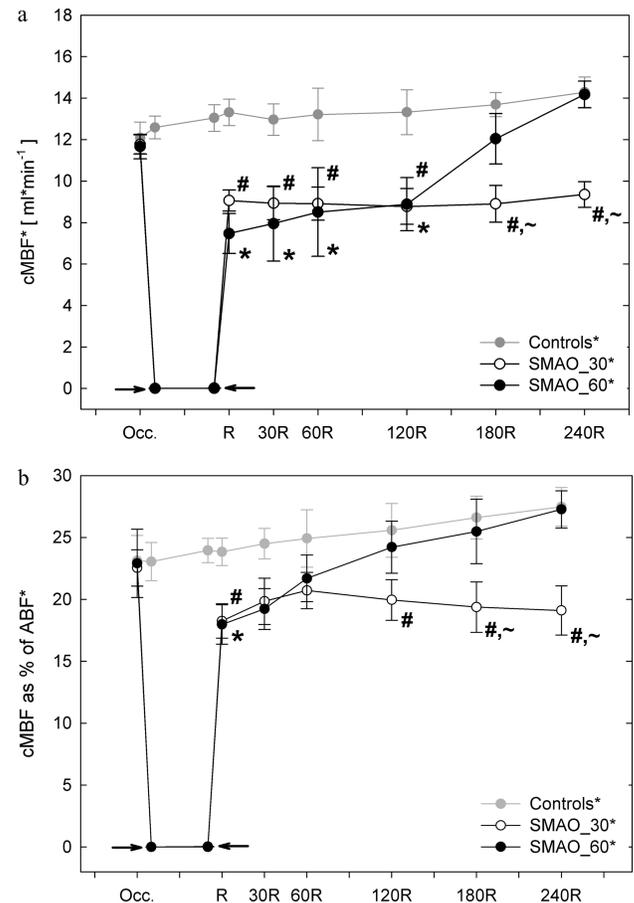


Figure 7. Continuous superior mesenteric artery blood flow measurements (cMBF)*, cMBF as % of ABF*. *Experiments with continuous SMA flow measurements and without intravital microscopy. Data are presented as means \pm SEM. SMAO_30: ischemia duration 30 min, SMAO_60: ischemia duration 60 min; Occ.: superior mesenteric artery occlusion; R, 30R, 60R, 120R, 180R, 240R: reperfusion (numbers indicate minutes). First arrow: Occ. + 10 min, second arrow: 10 min before R #Significant difference between SMAO_30* and controls*, $p < .05$; *significant difference between SMAO_60* and controls*, $p < .05$; ~significant difference between SMAO_30* and SMAO_60*, $p < .05$.

($p < .05$). In surviving animals in SMAO_60, base excess continued to decrease until the end of the experiment.

P_aO_2 values were different neither at baseline (Table 2) nor at R (data not shown). There was a trend to higher p_aO_2 values in ischemia groups during the course of the experiment as compared to controls. Hematocrit was comparable at baseline (Table 2). A transient increase in hematocrit was noted in SMAO_60 at R and in SMAO_30 at 60R (data not shown). Most likely due to the larger volume

Table 2. Selected parameters at baseline and 240R

	Baseline	120R	240R
pH			
Controls	7.37 ± 0.01	7.38 ± 0.01	7.37 ± 0.01
SMAO_30	7.39 ± 0.01	7.36 ± 0.02	7.33 ± 0.03
SMAO_60	7.38 ± 0.02	7.30 ± 0.03 ^{+,*~}	7.12 ± 0.09
P_aCO₂ (mm Hg)			
Controls	39.1 ± 2.1	36.2 ± 1.3	35.2 ± 0.8
SMAO_30	38.3 ± 0.9	32.2 ± 1.9 ^{+,#}	30.3 ± 4.4 ⁺
SMAO_60	39.4 ± 1.0	29.9 ± 2.3 ⁺	25.1 ± 3.7
P_aO₂ (mmHg)			
Controls	88.2 ± 3.8	81.1 ± 4.4	82.2 ± 3.8
SMAO_30	90.1 ± 5.3	94.9 ± 4.8	86.5 ± 9.5
SMAO_60	84.9 ± 3.5	86.2 ± 6.3	110.4 ± 18.5
Hct (%)			
Controls	45.4 ± 1.5	43.0 ± 1.1	40.5 ± 0.8 ⁺
SMAO_30	45.6 ± 1.3	46.3 ± 0.8	37.3 ± 1.6 ⁺
SMAO_60	45.8 ± 0.9	44.9 ± 2.2	32.6 ± 3.4
K⁺ (mmol/L)			
Controls	4.1 ± 0.3	4.2 ± 0.1	4.6 ± 0.3
SMAO_30	4.2 ± 0.1	4.6 ± 0.1	5.1 ± 0.3 ⁺
SMAO_60	4.4 ± 0.2	5.0 ± 0.3	6.0 ± 0.8
Na⁺ (mmol/L)			
Controls	140.4 ± 1.2	143.0 ± 0.9 ⁺	143.4 ± 1.0
SMAO_30	138.6 ± 0.5	141.0 ± 0.8 ⁺	143.4 ± 1.0 ⁺
SMAO_60	140.0 ± 1.1	141.2 ± 0.9	143.2 ± 1.5
Cl⁻ (mmol/L)			
Controls	106.2 ± 1.1	111.4 ± 0.5 ⁺	113.6 ± 0.7 ⁺
SMAO_30	104.2 ± 0.6	111.0 ± 0.9 ⁺	118.2 ± 1.5 ^{+,#}
SMAO_60	105.5 ± 1.0	117.3 ± 1.4 ^{+,*~}	126.2 ± 0.5

⁺Significant difference between 0 and 240R, $p < .05$.

[#]Significant difference between SMAO_30 and controls, $p < .05$.

^{*}Significant difference between SMAO_60 and controls, $p < .05$.

[~]Significant difference between SMAO_30 and SMAO_60, $p < .05$.

substituted in SMAO_60, hematocrit decreased in this group in surviving animals as compared to controls until 240R (Table 2).

Electrolytes, Selected Metabolites

Lactate levels did not differ at baseline (Figure 9), or until 120R. Lactate levels decreased in controls and SMAO_30 after 60R until the end of the experiments, whereas lactate levels remained constant in surviving animals in SMAO_60. There were no significant differences between the groups.

Serum potassium levels were comparable at baseline, and remained constant in controls (Table 2). Potassium increased significantly in SMAO_30 until the end of observation ($p < .05$), and increased in SMAO_60 in surviving animals until 240R (Table 2). Potassium levels were not significantly different between SMAO_30 and controls at 240R.

Sodium levels tended to increase in controls and increased significantly ($p < .05$) in SMAO_30 and SMAO_60 from baseline to 120R, and in SMAO_60 from baseline to 240R (Table 2). However, there were no statistically significant differences in sodium

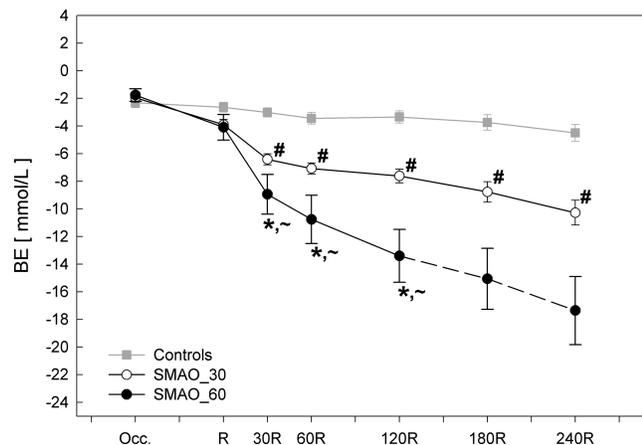


Figure 8. Base excess (BE). Data are presented as means ± SEM. SMAO_30: ischemia duration 30 min, SMAO_60: ischemia duration 60 min; Occ.: superior mesenteric artery occlusion; R, 30R, 60R, 120R, 180R, 240R: reperfusion (numbers indicate minutes). [#]Significant difference between SMAO_30 and controls, $p < .05$; ^{*}significant difference between SMAO_60 and controls, $p < .05$; [~]significant difference between SMAO_30 and SMAO_60, $p < .05$. Data from SMAO_60 at 180R and 240R were not compared to the other groups with a statistical test due to a significantly reduced group size. These time points are connected to the previous time point with a dashed line.

levels between all groups (Table 2). Serum chloride levels were comparable at baseline, and increased significantly ($p < .05$) in all groups from R to 120R (Table 2). Serum chloride levels differed

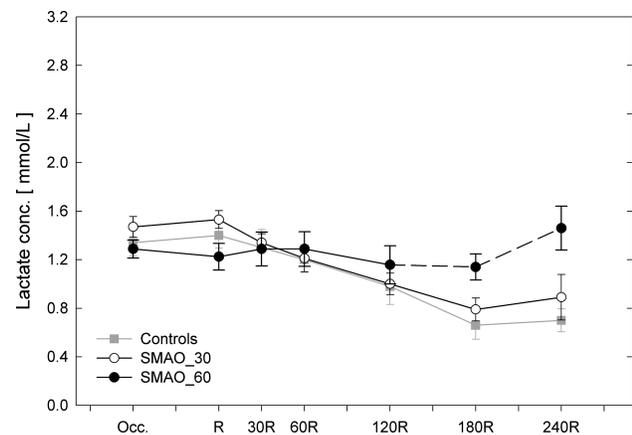


Figure 9. Lactate concentration. Data are presented as means ± SEM. SMAO_30: ischemia duration 30 min, SMAO_60: ischemia duration 60 min; Occ.: superior mesenteric artery occlusion; R, 30R, 60R, 120R, 180R, 240R: reperfusion (numbers indicate minutes). Data from SMAO_60 at 180R and 240R were not compared to the other groups with a statistical test due to a significantly reduced group size. These time points are connected to the previous time point with a dashed line.

significantly ($p < .05$) between controls, SMAO_30, and SMAO_60 at 120R, and between SMAO_30, and controls at 240R (Table 2). Surviving animals in SMAO_60 showed a further increase in serum chloride levels until the end of the experiments.

DISCUSSION

In current experiments, shunting of the microcirculation was observed after 60 minutes of superior mesenteric artery ischemia followed by 4 h of reperfusion. The term shunting is commonly used to describe a perfusion heterogeneity in which arterial blood flow is shortcut before reaching the level of small vessels and capillaries in tissues, and is usually discussed in the context of sepsis and septic shock (20–22). However, shunting of the microcirculation has not been described after mesenteric ischemia and reperfusion, and was not observed in our study immediately after reperfusion. Our previous study with superior mesenteric artery (SMA) flow measurements revealed macrocirculatory flow patterns as usually seen in sepsis and septic shock after 60 min of superior mesenteric artery occlusion followed by 4 h of reperfusion (14). In this previous study, blood flow in the superior mesenteric artery returned to baseline values and peripheral resistance normalized until the end of the observation period, whereas metabolic deterioration continued and mortality was increased (14). In the current study, SMA blood flow recordings were obtained at baseline and at the end of the experiments. Further, experiments with continuous SMA blood flow measurements were performed with these data confirming the results of our previous study (14). SMA blood flow was lower after reperfusion in both ischemia groups compared to controls and remained on this level only in animals subjected to 30 min of ischemia. As shown in the previous study, reduced mesenteric blood flow in animals subjected to 30 min of ischemia, which persists until the end of the experiments, is attributable to an increased vascular resistance in the superior mesenteric artery-dependent vasculature (14). After 60 min of ischemia, SMA blood flow increased during late reperfusion to comparable levels as in control animals. In relation to abdominal blood flow, SMA blood flow remained constant after reperfusion in animals subjected to 30 min of SMA occlusion and controls, whereas it increased in animals undergoing 60 min of ischemia until the end of the experiments. Hence, the absolute increase in SMA blood flow in animals with 60 min occlusion period cannot be explained by the recovery of abdominal blood flow in these

animals with time. With this pattern of changes in both micro- and macrocirculation, the current study strongly supports the hypothesis of shunting of the microcirculation raised previously (14).

Prolonged ischemia (60 min) had significant impact on survival, macrocirculation, and obtained blood gases/metabolic parameters. The survival rate was significantly reduced depending on the duration of ischemia, which is consistent with clinical findings (16). Compared to animals subjected to exteriorization of an ileal loop, animals in experiments with continuous mesenteric blood flow measurements and without exteriorization of an ileal loop had a significantly reduced mortality despite comparable ischemia times. Since the only difference in the setup between these two sets of experiments was the exteriorization of an ileal loop, the higher death rate is most likely attributable to the additional trauma to the tissue by this procedure resulting in an enhanced systemic inflammatory response [12].

Red blood cell velocity is usually significantly reduced after SMA ischemia/reperfusion, as shown in previous experiments [14], and described by others [2, 3]. There is evidence that thrombotic microvascular obstruction is at least partially responsible for the reduced perfusion of the SMA-dependent circulation after ischemia [19]. In the present study, mesenteric microcirculatory perfusion usually returned at a reduced level as compared to baseline after release of the occluding snare. However, this return of microcirculatory perfusion was of only short duration and microcirculatory perfusion deteriorated rapidly to low-flow/no-flow conditions in animals subjected to 60 min of ischemia. The number of rolling and adherent leukocytes increased shortly after reperfusion in both ischemia groups. The increase in the number of rolling leukocytes was only transient, whereas adherent leukocytes remained at a high level in the group undergoing 30 min of ischemia, and decreased from there transiently after reperfusion in the group subjected to 60 min of ischemia. The transient reduction of adherent leukocytes in this group might be explained by the reduced leukocyte recruitment to vessels with minimal flow. Since the recruitment of leukocytes was completely abrogated in vessels with persistent no-flow, leukocyte counts in these vessels were excluded from the analyses. This is because a clear distinction between rolling and adherent leukocytes depends on flow.

The amount of intravenous fluids required to maintain mean arterial blood pressure increased significantly with the duration of ischemia as described by

others [4]. Animals subjected to 60 min of ischemia required an 8-fold larger volume of intravenous fluids compared to animals with 30 min of ischemia. Even though all animals received intravenous fluid replacement during reperfusion according to the experimental protocol (to maintain MAP > 70 mm Hg), blood pressure decreased from baseline values beginning after reperfusion and dependent on the duration of superior mesenteric artery ischemia. The decrease in mean arterial blood pressure was accompanied by increased heart rates and preceded by a reduction in abdominal blood flow. The changes in abdominal blood flow were most prominent in the group subjected to 60 min of ischemia and started during occlusion of the superior mesenteric artery. Abdominal blood flow remained lower in ischemia groups despite the circulatory support provided by means of intravenous fluid replacement. Interestingly, surviving animals in the group subjected to 60 min of ischemia did not need extraordinary large total volumes (45–56 mL) compared to some of the nonsurvivors (127, 154 mL). In nonsurviving animals, the total volume applied to an animal correlated positively (data not shown) with the overall surviving time of the individual animal. Animals that survived longer within the observation period, in general, required larger total volumes. Since typically only surviving animals showed a recovery of abdominal blood flow until the end of the experiments, the infused total volume alone does not account for the recovery of abdominal blood flow in this group.

The microcirculatory and macrocirculatory changes were paralleled by changes in blood gas values and metabolic parameters. Metabolic acidosis was seen in both ischemia groups, the degree of metabolic acidosis correlating with the duration of ischemia. Animals hyperventilated to a similar extent in ischemia groups. In animals subjected to 30 min of ischemia hyperventilation partially compensated metabolic acidosis and maintained blood pH on similar levels as in controls. However, in animals undergoing 60 min of ischemia, respiratory compensation failed and acidosis continued to worsen until the end of observation. Interestingly, a transient increase in hematocrit was seen in ischemia groups, the temporal appearance matching to some extent to the severity of ischemic damage. This transient increase in hematocrit was noted in animals subjected to 60 min of ischemia at reperfusion, whereas this change in hematocrit appeared in animals subjected to 30 min of ischemia at 60 min after reperfusion (data not shown). The decrease in abdominal blood

flow together with the progressive metabolic acidosis and the transient increase in hematocrit might be due to an insufficient fluid management in our experiments, since there is evidence that there is a positive correlation between aggressive fluid therapy and improved outcome after mesenteric ischemia (17,18). However, our fluid replacement strategy aimed at mimicking typical clinical situations, where fluid replacement is usually blood pressure guided. Further, excessive fluid therapy is problematic in clinical situations as it bears a risk of pulmonary edema in the mostly elderly patient population at risk of mesenteric ischemia (1). The fluid management in our experiments did not have any noticeable effect on oxygenation (p_aO_2), nor did the observed changes in macrocirculation and blood gases. As a consequence of the relatively large volumes of normal saline used for fluid replacement, serum chloride levels increased significantly after 60 min of ischemia as compared to controls from the beginning of reperfusion until 120R, and continued to rise in surviving animals until the end of the experiments. In animals subjected to 30 min of ischemia, there was only a trend to higher chloride levels. Hyperchloremia might contribute to metabolic acidosis, and might theoretically worsen outcome, although this has never been clearly shown (15). However, hypovolemia and shock do promote metabolic acidosis as well. In a system with intact counter-regulatory mechanisms, the contribution of hyperchloremia to metabolic acidosis might be less important, and saline infusion might actually attenuate blood pH (17).

Blood lactate is not considered a reliable marker of mesenteric ischemia, since increases in lactate levels are nonspecific and lactate produced in the upper gastrointestinal tract is usually rapidly metabolized by the liver before entering systemic circulation (7,11). Confirming previous experiments, blood lactate concentration did not increase in response to superior mesenteric artery ischemia (14). Further, blood lactate concentration does not necessarily reflect lactate concentration in ischemic tissues as no-flow might prevent lactate from being washed out into systemic circulation. In our experiments, lactate production remained at baseline values in the group subjected to 60 min of ischemia in surviving animals, while it decreased with time in animals subjected to 30 min of ischemia and in control animals. Potassium levels tended to increase in both ischemia groups as compared to controls. This increase in potassium levels might be explained by release of large amounts of potassium into systemic circulation through

disintegrated membranes of significant numbers of necrotic cells (25).

The observed changes in the recorded local and global parameters indicate that ischemic damage might be persistent after a certain threshold of ischemia duration. Blood flow did recur in ileal arteries upstream and in ileal venules downstream to the recorded areas, whereas no-flow persisted in small vessels within recorded areas in animals subjected to 60 min of ischemia. These observations are most likely caused by shunting of the microcirculation, even though we could not quantify the shunt volume with our experiments.

Diagnosis and treatment of mesenteric ischemia in clinical settings remain challenging tasks, as methods to diagnose this uncommon but serious clinical problem are still limited (16). Therefore, the diagnosis is often delayed and mortality is still high (5). Our superior mesenteric artery occlusion model might provide useful insight into perfusion heterogeneities as seen in mesenteric ischemia and conditions such as sepsis and septic shock (23). Further, it might especially be helpful to evaluate therapeutic modalities that influence mesenteric microcirculation and outcome. Shunting of the microcirculation might significantly contribute to the still poor prognosis of mesenteric ischemia, since any systemic treatment might not reach postischemic tissue after a certain “dose” of ischemia due to shunting of the microcirculation. Experimental and clinical strategies to treat mesenteric ischemia should consider shunting of the microcirculation after mesenteric ischemia as a significant confounder, which needs to be addressed in the attempt to improve the still poor prognosis of mesenteric ischemia.

CONCLUSION

SMA occlusion for 60 min and subsequent reperfusion lead to perfusion abnormalities in the mesenteric microcirculation as often seen in sepsis and septic shock, with increased shunting of the microcirculation, progressive metabolic acidosis, and increased mortality. To detect these significant changes requires prolonged observation periods and might help to find new treatments to improve the still poor prognosis of mesenteric ischemia.

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