Microcirculatory heterogeneity during compromised flow conditions in the intestine

Summary of Ph. D. thesis

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Kecskemét, Hungary

2004

Szeged

I. INTRODUCTION AND AIMS

The main goal of the studies was to investigate the consequences of systemic circulatory disorders (hemorrhagic shock, endotoxemia, nitric oxide synthesis inhibition) and local perfusion disturbances (ischemia-reperfusion) on the small intestinal microcirculation. Although there is now a general consensus regarding the major role of mucosal microcirculatory dysfunction in the pathophysiology of low-flow states, important questions remain to be resolved. Firstly, microcirculatory analysis is difficult in all tissue types when perfusion heterogeneity is present. Secondly, the conventional parameters of spatial heterogeneity (e.g. functional capillary density) and timewise heterogeneity (i.e. cycles/min) are insensitive to trace subtle microcirculatory alterations. Further, the comparison of velocities between continuous flow and pulsatile perfusion phases, between fast and slow-flow transition and between different flow patterns is impossible by these means.

The aims were to devise a method of data analysis which takes into consideration all major components of flow variability and characterize the statistical properties of oscillatory flow in the microcirculation. For this reason, a new mathematical approach was developed which takes the changes in amplitude and duration simultaneously into account. Using the novel formula, three major forms of intramural heterogeneity could be distinguished: **1.** timewise, **2**. between anatomic layers, and **3.** within-layer heterogeneity.

Our next purpose was to define the consequences of vasoactive therapies targeting the intestinal microcirculation. To this aim we compared the effectiveness of different treatment modalities (physiologic saline, hypertonic-hyperosmotic resusctitation, endothelin–A receptor antagonism) in experimental hemorrhagic shock.

II. MATERIALS AND METHODS

Hemodynamic measurements

Rat studies

The mean arterial blood pressure (MAP) was recorded, the microcirculation of the mucosal villi and the longitudinal muscle was continuously visualized by intravital videomicroscopy with the OPS imaging technique (CYTOSCAN A/R, Cytometrics, Inc. PA, USA). The device provides an optimal imaging of hemoglobin-containing microvascular structures for intravital microscopy at a chosen focus level (Groner et al *Nat Med.* 5, 1209-1212. 1999). Videomicroscopic images were recorded intermittently with an S-VHS videorecorder (Panasonic AG-MD 830) and quantitative assessment of the microcirculatory parameters was performed off-line by frame-to-frame analysis of the videotaped images. The functional capillary density (FCD), the length of the perfused capillaries per observation area (cm/cm^2) of the villi and the capillary red blood cell velocity (RBCV) (μm/s) were determined by means of a computer-assisted image analysis system (IVM Pictron, Budapest, Hungary).

Dog studies

In canine studies, MAP, pulmonary arterial pressure, and central venous pressures were measured. Cardiac output was determined, normalized for body weight and expressed as cardiac index (CI, ml/kg/min). The microcirculation of intestinal villi was visualized using the OPS imaging technique (see above). During capillary flowmotion, RBCV was determined during high flow and low (or stop) flow conditions.

Experimental protocol

Rat studies

The microirculatory variables were determined in 5 groups. Group 1 (n=6) served as sham-operated control. In other animals, blood was withdrawn from the femoral artery until the MAP reached 50 mmHg, and this level was maintained for 60 min. In group 2 (n=6) fluid replacement was conducted with 0.9% saline (200% of the shed blood volume), followed by 15

ml/kg/hr lactated Ringer's solution, in Group 3 (n=6) with 7.2% NaCl-10% HES 200/05 (Osmohes®, Fresenius Kabi GmbH, Graz, Austria). The microcirculatory changes were observed for 120 min in the resuscitation period. In group 4 (n=6), the superior mesenteric artery was isolated and clamped for 30 min which was followed by 120 min reperfusion. The animals in group 5 (n=6) received endotoxin infusion (*E. coli* 055:B5, 10 mg/kg/h, Sigma, St. Louis, MO) for 120 min. In group 6 (n=6) non-specific nitric oxide (NO) synthesis inhibition was induced with 10 mg/kg of N-ω-nitro-L-arginine methyl ester (L-NAME, Sigma, St. Louis, MO) iv. over 5 min, and the microcirculatory changes were observed for 120 min.

Canine studies

The animals were randomly assigned into 4 groups. Group 1 served as sham-operated control (n=3) while groups 2-4 were subjected to HS. Baseline variables were recorded for 30 min, then blood was withdrawn until the MAP reached 40 mmHg and this was maintained for 60 min. The animals were monitored for 180 min after HS.

In Group 2 (n=11), the animals were resuscitated with 0.9% saline (150% of the lost blood volume) over 10 min, followed by a low-rate infusion of saline (1 ml/kg/hr). Group 3 (n=10) was treated with hypertonic saline-dextran solution (HSD, 7.2% NaCl-10% dextran, 4 ml/kg,) over 10 min, followed by a continuous infusion of saline (1 ml/kg/hr). The animals in Group 4 (n=8) were treated with the selective ET-A receptor antagonist ETR-p1/fl peptide (100 nmol/kg iv. bolus in 1.5 ml/kg saline) 5 min before resuscitation and then HSD (4 ml/kg over 10 min) and saline infusion (1 ml/kg/hr) was given.

III. RESULTS AND DISCUSSION

Macrohemodynamic changes

In rat studies no significant changes in MAP were observed in sham-operated animals. HS was induced by withdrawing similar volume of blood in both experimental groups. During resuscitation, MAP did not reach baseline in the saline-resuscitated group (baseline: 105±3 mmHg, t=15 min: 86±5 mmHg, $p<0.001$), but it was rapidly restored and maintained for 60 min after Osmohes (baseline: 96±6 mmHg, t=15 min: 106±9 mmHg, t=60 min: 90±11 mmHg) which was followed a gradual decrease in both group. In response to NO synthesis inhibition, an immediate, approximately 30% rise in MAP (compared to the 124±2 mmHg baseline) was detected, and the elevation persisted until the end of the experiment. Endotoxin infusion and ischemia-reperfusion did not influence the MAP significantly.

In the second series of studies in dogs the macro- and microhemodynamic parameters did not change significantly during the 330 min observation period. In HS, the approximately 50% reduction of the calculated blood volume was accompanied by an approx. 70% decrease in CI. Resuscitation was followed by a partial recovery in MAP, irrespective to the employed therapy.

Microhemodynamic changes

Heterogeneity in time – flowmotion

In sham-operated rats, the microhemodynamic parameters did not change significantly over time and the microcirculatory perfusion was always continuous in both structures (RBCV was 606±6 μm/s in the villi, and 618±5 μm/s in the muscle layer). HS induced a time-dependent fluctuation in flow in the villus capillaries, with an alternating sequence of high (~380-440 μm/s) and low (~120-140 μm/s) velocity. The flow pattern displayed a square wave character and not a sinusoid, as the initiation and cessation of flow were abrupt. Accordingly, the durations of the different periods could be accurately measured. The distribution of high- and low-flow periods was reasonably uniform within a given time frame; thus the oscillatory pattern could be

characterized by the weighted average of the RBCVs (A-RBCV) with the relative durations used as weighting factors.

$$\frac{V_1T_1 + v_1t_1 + V_2T_2 + v_2t_2 + \Lambda\ V_nT_n + v_nt_n}{T_1 + t_1 + T_2 + t_2 + \Lambda\ T_n + t_n} \qquad \textbf{(1)}$$

In this formula, V_n and T_n are the RBCV and the relative duration referring to the high-flow periods, while v_n and t_n are the RBCV and relative duration referring to the low-flow periods.

The results of the above calculations indicated that a decrease of approximately 40% in A-RBCV occurred in the villi during hemorrhage. At resuscitation onset, continuous flow periods were again present in a majority of the experiments (4/6), but the A-RBCV (and RBCV) did not reach the baseline level. Hypoperfusion in the villi, however, could only be partially eliminated by saline and flow-motion reappeared in every villus after 30 min of resuscitation. Osmohes completely restored A-RBCV by increasing both red blood cell velocity values and the duration of high flow periods at the onset or resuscitation in the villi.

In other rat studies, intestinal ischemia-reperfusion resulted in a persistent, uniform, approximately 20% A-RBCV decrease in both structures. The microcirculation was continuous, and variability in time was not observed. Endotoxin infusion was associated with a significant reduction in A-RBCV (20% and 25% decrease in the mucosa and muscle layer, respectively), heterogeneity in time was not detected. NO synthase inhibition by L-NAME was accompanied by a uniform, more than 40% reduction in A-RBCV in both layers.

In dogs, microcirculatory flow was continuous at the villus tips under control conditions, while cyclic fluctuation appeared during hemorrhage and the above calculation was used again to calculate A-RBCV. At the onset of resuscitation continuous flow periods were transiently seen in 33%, 40% and 50% of the experiments after saline, HSD, and HSD + ETR p1/fl treatment, respectively. During the later stages of resuscitation, the relative duration of high RBCV periods was decreased, indicating the predominance of oscillatory flow in the villi. The ET-A receptor antagonism significantly increased the relative duration of the high RBCV periods at the onset of

resuscitation either by prolonging the continuous flow or the duration of high flow periods during oscillation

Heterogeneity in space

In sham-operated rats, the FCD did not change significantly during the observation period, and spatial heterogeneity in villus or muscle perfusion was not detected. Hypovolemia in rats induced flowmotion in the villi; the FCD was therefore determined during the high-flow phases of oscillation. With this restriction, HS was not associated with changes in villus FCD, but it gradually decreased (by 30%) in the late phase of resuscitation. Severe perfusion heterogeneity was also observed in the longitudinal muscle. Here, capillaries with complete stasis were not identified and therefore the FCD did not change significantly.

During hemorrhagic hypotension in rats, the relative length of high-velocity capillaries accounted for 42-70% (66% on average) of the total capillary network in the muscle layer. Since both the RBCV and the relative length of the capillaries representing these velocities change dynamically over time, calculation of the arithmetic average RBCV from randomly chosen capillaries cannot give a reliable measure of the actual perfusion condition. For this reason, the A-RBCV was calculated again, using the relative lengths of the capillaries as weighting factors.

$$\frac{\mathrm{VL} + vl}{\mathrm{L} + l} \qquad \textbf{(2)}$$

where V and L are the average RBCV referring to high-flow areas and the relative length of the capillaries in the corresponding area, respectively, while *v* and *l* are the average RBCV and the corresponding relative length of the capillaries referring to the low-flow areas, respectively.

Accordingly, A-RBCV decreased by 60-70% in the longitudinal muscle during HS. During resuscitation with saline, perfusion heterogeneity was not eliminated completely, and the length of the hypoperfused part of the capillary network then gradually increased (up to approximately 55%). Therefore, after a partial recovery during the early phase of resuscitation, the A-RBCV gradually deteriorated during resuscitation. The resuscitation onset with Osmohes

was characterized by similarly modest degree of recovery in muscle mircrovascular perfusion, but it resulted in higher A-RBCV values compared saline treatment.

In other groups with ischemia-reperfusion, the villus FCD was decreased by approximately 20% after 30 min of reperfusion and significant spatial heterogeneity was not seen in the muscle layer. Endotoxin infusion resulted in a significant decrease in villus FCD after 15 min, which was followed by further 20% decline at 30 min. Thereafter, this value persisted throughout the examined period in the villi, without any significant change in muscle FCD.

The non-specific NO synthase inhibition was accompanied by an immediate, significant and persisting FCD reduction (ranging between 40 and 60%) in the villi, but no spatial heterogeneity (or FCD change) occurred in the muscle layer. Additionally, alternating (on-off) flow evolved within adjoining villi, i.e. some villi were perfused with a higher, and others with a characteristically lower RBCV within the observation field throughout the given time frame (90 s). An average calculation (A-RBCV) that considers the spatial distribution of RBCV was again used, where the area representing a certain velocity range was used as weighting factor. The A-RBCV in the mucosal villi was decreased by 40% after NO synthase inhibition.

During HS in dogs, lack of perfusion in capillaries was not observed under control conditions or during oscillation, hence villus FCD was unchanged. Resuscitation was not associated with obvious signs of capillary plugging, but an increase in intercapillary distance was recognized in saline-treated animals.

IV. SUMMARY OF NEW FINDINGS

1. Using a novel probabilistic approach, continuous and oscillatory flow states as well as spatial heterogeneity can be compared and quantitatively expressed.
2. Three major forms of intramural microcirculatory heterogeneity could be distinguished in the small intestine: **a.** heterogeneity between anatomic layers (intramural redistribution of microcirculatory flow), **b.** within-layer heterogeneity, and **c**. time-wise heterogeneity (oscillatory flow pattern).
3. Resuscitation with hypertonic-hyperoncotic solution (Osmohes) in rats caused an increased duration of high-flow phases during flowmotion which represents a sign of improved tissue perfusion. The positive effect of ET-A receptor antagonism was also manifested in a significantly increased relative duration of high RBCV periods at the onset of resuscitation in dogs.
4. A good correlation between the microcirculatory improvement and the reduction of heterogeneity can be demonstrated in the small intestine. Variability in velocity should be taken into account for the quantitative analysis of the microcirculation. A-RBCV provides comparative measure of continuous and oscillatory flow periods, and should be used to assess the efficacy of different resuscitation strategies at the microcirculatory level.

ACKNOWLEDGMENTS

I am grateful to Professors Sándor Nagy and Mihály Boros for the opportunity to work in the Institute of Surgical Research and for their valuable scientific guidance and help.

I am indebted to Dr. Andrea Szabó who helped me greatly to learn the necessary surgical and experimental skills. Special thanks are due to all the technical staff of the Institute. Their skilful help and enthusiastic work have helped me overcome many difficulties and have facilitated the experiments. I also thank to all of my colleagues for their contributions to my experimental work.

List of full papers related to the subject of the thesis

I. **Kornél Vajda**, Andrea Szabó, Katalin Kucsa, Béla Suki, Mihály Boros: Microcirculatory heterogeneity in the rat small intestine during compromised flow conditions. Microcirculation 2004 (accepted, in press).

II. Andrea Szabó, Béla Suki, Endre Csonka, Edgár Eszlári, Katalin Kucsa, **Kornél Vajda**, József Kaszaki, Mihály Boros: Flowmotion in the small intestinal villi. A new method to characterize the microcirculation. Shock 2004 (accepted, in press).

III. **Vajda Kornél,** Szabó Andrea, Boros Mihály: A vékonybél mikrokeringésének jellemzése helyi és általános keringési zavarokban. Magyar Sebészet 2003, 56:80-85.

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V. **Kornél Vajda**, Andrea Szabó, Mihály Boros: Osmohes reduces microcirculatory heterogeneity in the rat small intestine during resuscitation from hemorrhagic shock. Eur Surg Res (submitted for publication).

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I. **Vajda K,** Szabó A, Sárkány A, Kucsa K, Thoman A, Boros M: Generalized and local circulatory disorders differentially affect the microcirculation of the different layers of the rat small intestine. Eur Surg Res 34 (S1): 5-6, 2002

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