Inhaled Nitric Oxide with Intravenous Hydrocortisone Does Not Improve Microvascular Perfusion in Septic Pigs.

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Research Article

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Abstract

Background

Capillary flow restoration in sepsis may prevent organ dysfunction caused by a prolonged impairment of microvascular flow. The main aim of the study was to investigate the microcirculatory effect of inhaled nitric oxide (iNO) combined with intravenous hydrocortisone in a porcine model of sepsis. The second aim was to evaluate the influence of hemodynamic resuscitation with noradrenaline and crystalloids on capillary flow.

Materials and Methods

In the study, 11 piglets of Polish breed were generally anaesthetized and underwent surgical colon perforation. The animals were randomly allocated to one of three treatment groups. Group 1 received iNO and hydrocortisone, whereas Group 2 was a control group. Both groups were resuscitated with crystalloids and noradrenaline if hypotensive. Group 3 received no treatment at all.

In a 30-hour period of observation, we assessed microcirculation using sidestream dark field imaging (SDF) and monitored hemodynamics with a pulmonary artery catheter.

Results

We found no effect of iNO with hydrocortisone on microcirculation in septic piglets. Fluid and vasopressor treatment postponed microvascular flow impairment and led to a higher microcirculatory flow index, greater proportion of perfused vessels, and perfused vessel density.

Conclusion

Crystalloid and vasopressor treatment postpones microvascular flow derangement and organ dysfunction in septic piglets.

Introduction

Every year, 31 million people are hospitalized because of sepsis, and over 5 million die with this diagnosis [1]. For this reason, early detection and treatment of sepsis have been of great interest to intensivists.

Sepsis and its complications including multiorgan failure seem to result from changes in capillary perfusion, which may precede more severe symptoms [2, 3]. It has been proven that persistent microcirculatory derangement is strongly related to poor outcome and can be observed in patients with multiorgan dysfunction even after shock resolution [4, 5]. Therefore, capillary flow monitoring appears to be essential for early and successful management of sepsis.

There are multiple studies concerning septic patients and septic animal models that provide reproducible information about microcirculation changes occurring in sepsis [6–8]. Various processes activated by

inflammation lead to the cessation of flow in some capillaries and augmentation of flow in others [9]. An increased distance between perfused capillaries impedes oxygen diffusion and its delivery to cells, creating microcirculatory shunt [10]. It leads to oxygen deficit in tissues, increased lactate production, shock, and, finally, organ dysfunction [2, 11, 12].

Microvascular monitoring is crucial not only for early detection of pathologies but it has also been used to assess the effects of treatment with multiple drugs e.g. diuretics, hydrocortisone, phenylephrine, nitroglycerin, or red blood cell transfusion [13–16].

Therapies tested in sepsis (e.g. corticosteroids) modulate the inflammatory response. The use of hydrocortisone in septic shock seems beneficial not only due to its anti-inflammatory properties, since glucocorticoids inhibit pro-inflammatory gene expression and activate anti-inflammatory protein production [17]. It also increases vessel responsiveness to catecholamines [18]. Moreover, moderate doses of hydrocortisone administered in early stages of septic shock improve capillary perfusion [13]. The role of corticosteroids in the management of septic shock is still controversial. Nevertheless, low doses of corticosteroids may restore circulation and reduce mortality caused by septic shock [13, 19, 20].

As far as sepsis treatment is concerned, much attention has also been paid to nitric oxide (NO) with multiple trials targeting both NO production inhibition and external NO delivery [21]. Generally, the release of nitric oxide is increased in septic shock. However, in some areas NO production may be impaired, which is one of the factors leading to heterogeneity and hypoperfusion of the microvascular bed [12, 21, 22]. The delivery of exogenous NO, a very potent vasodilator, might potentially resuscitate microcirculation and restore tissue perfusion [21, 23]. Inhaled nitric oxide (iNO) is one of the donors tested not only because of its pulmonary activity, but also because of recognized peripheral effects [24]. Experimental models of sepsis revealed that inhaled nitric oxide up-regulates glucocorticoid receptors and blunts the inflammatory reaction [25].

For the above reasons, our objective was to assess the effect of iNO and hydrocortisone on microcirculatory flow in a porcine septic model. The aim of the study was to verify the theory that iNO and hydrocortisone administered in sepsis preserve microvascular flow. We also wished to test whether fluid resuscitation combined with vasopressors resuscitates microcirculation. For microvascular flow assessment, we applied sidestream dark field imaging described elsewhere [26].

Results

All eleven animals developed SIRS (Systemic Inflammatory Response Syndrome) (Table 1). There were no differences between the three studied groups as far as initial microcirculatory parameters are concerned (Fig. 2).

Table 1
Group characteristics demonstrating the number of animals at particular time points, number of animals that developed SIRS, hemodynamic parameters, and temperature (expressed as means ± SD).

	Group	START	20 h	30 h
No of animals	1.iNO + hydrocortisone	4	4	3
	2.Control	4	3	3
	3.No treatment	3	3	0
No of animals	1.iNO + hydrocortisone	-	4	3
With SIRS	2.Control	-	3	3
	3.No treatment	-	3	-
MAP [mm Hg]	1.iNO + hydrocortisone	70 ± 21	85 ± 31	82 ± 6
	2.Control	74 ± 7	76 ± 21	72 ± 23
	3.No treatment	74 ± 5	45 ± 9	
SAP [mm Hg]	1.iNO + hydrocortisone	112 ± 10	127 ± 47	120 ± 7
	2.Control	100 ± 13	124 ± 23	98 ± 17
	3.No treatment	93 ± 2	76 ± 14	
DAP [mm Hg]	1.iNO + hydrocortisone	65 ± 4	67 ± 29	61 ± 8
	2.Control	61 ± 9	67 ± 18	44 ± 12
	3.No treatment	60 ± 9	26 ± 4	
MPAP [mm Hg]	1.iNO + hydrocortisone	25 ± 5	22 ± 3	16 ± 6
	2.Control	20 ± 4	31 ± 6	20 ± 7
	3.No treatment	15 ± 4	21 ± 7	
SPAP [mm Hg]	1.iNO + hydrocortisone	33 ± 5	27 ± 3	22 ± 2
	2.Control	29 ± 9	33 ± 6	26 ± 11
	3.No treatment	20 ± 5	24 ± 6	
DPAP [mm Hg]	1.iNO + hydrocortisone	20 ± 7	19 ± 3	13 ± 8
	2.Control	17 ± 7	28 ± 5	17 ± 6
	3.No treatment	11 ± 13	19 ± 6	
CVP [mm Hg]	1.iNO + hydrocortisone	13 ± 2	15 ± 6	7 ± 4

	Group	START	20 h	30 h
	2.Control	12 ± 3	15 ± 6	13 ± 7
	3.No treatment	5 ± 2	4 ± 1	
PCWP [mm Hg]	1.iNO + hydrocortisone	14 ± 3	15 ± 4	14 ± 5
	2.Control	12 ± 1	19 ± 3	12 ± 2
	3.No treatment	8 ± 6	16 ± 12	
CO [L/min]	1.iNO + hydrocortisone	2.0 ± 0.6	3.7 ± 1.7	3.6 ± 1.5
	2.Control	2.0 ± 0.9	3.0 ± 0.9	2.8 ± 0.5
	3.No treatment	1.9 ± 0.3	4.2 ± 0.6	
CI [L/min/m2]	1.iNO + hydrocortisone	3.3 ± 1.0	6.1 ± 2.9	6.1 ± 2.5
	2.Control	3.4 ± 1.0	5.0 ± 1.4	4.7 ± 0.9
	3.No treatment	3.0 ± 0.5	6.7 ± 0.9	
SVI [ml/m2]	1.iNO + hydrocortisone	43 ± 12	45 ± 23	40 ± 17
	2.Control	43 ± 22	38 ± 8	35 ± 10
	3.No treatment	36 ± 5	43 ± 6	
SVRI [dyn x s/cm5]	iNO + hydrocortisone	1636 ± 347	1217 ± 1062	1111 ± 438
	Placebo	1754 ± 943	1033 ± 338	910 ± 242
	No treatment	1723 ± 385	501 ± 151	
Temperature [°C]	iNO + hydrocortisone	36.0 ± 0.6	41.3 ± 0.5	41.5 ± 0.6
	Placebo	36.7 ± 0.6	41.0 ± 0.6	41.9 ± 0.7
	No treatment	37.5 ± 0.8	41.5 ± 0.7	

The 30-hour analysis showed no influence of iNO and hydrocortisone on microvascular bed perfusion. In both groups, microvascular flow index (MFI), small vessel microvascular flow index (sMFI), perfused vessel density (PVD), perfused small vessel density (sPVD), proportion of perfused vessels (PPV), and proportion of perfused small vessels (sPPV) significantly dropped. There were no marked intergroup differences in perfusion impairment (Table 2).

Table 2. Table demonstrating changes of the TVD, sVD, and de Backer score over time. At all time points, there were no significant differences in the above variables between the studied groups (p>0.05). The TVD, sVD, and de Backer score did not change over time. The data are presented as medians (25th–75th percentiles).

The statistical analysis of HI (mean ± SD) was not possible due to the small size of the analyzed groups.

Group	START	*p*	20 hours	*p*	30 hours	*p*
TVD [mm/mm2]						
iNO+hydrocortisone	17.4 (16.8-19.0)	*0.35*	16.3 (15.7-18.4)	0.33	17.0 (13.4-19.1)	0.08
Control	18.6 (17.4-21.3)		18.6 (15.4-19.7)		18.4 (16.6-21.0)	
No treatment	18.0 (16.0-20.3)		17.3 (14.3-19.0)		-	
sVD [mm/mm2]						
iNO+hydrocortisone	16.0 (14.7-18.5)	*0.30*	15.5 (14.6-16.4)	0.47	16.5 (11.9-17.4)	0.09
Control	17.4 (16.0-19.9)		15.8 (14.3-17.3)		16.9 (14.8-19.5)	
No treatment	16.7 (15.3-19.5)		15.3 (13.2-17.6)		-	
DeBacker score [1/mm]						
iNO+hydrocortisone	12.0 (10.5-13.1)	0.66	11.0 (9.7-12.8)	0.74	11.3 (9.4-14.4)	0.78
Control	12.4 (10.8-13.6)		11.2 (9.1-13.5)		12.7 (11.3-13.7)	
No treatment	11.8 (10.8-13.4)		11.6 (10.5-13.4)		-	
HI						
iNO+hydrocortisone	0.21 ± 0.13	n/a	0.24 ± 0.07	n/a	0.31 ± 0.02	n/a
Control	0.25 ± 0.23		0.22 ± 0.13		0,74 ± 0.77	
No treatment	0.37 ± 0.40		1.09 ± 0.83		-	

Group 3 died prematurely (after 12, 23 and 23.5 hours). For this reason, the complete analysis of all groups could only be conducted at 20 hours after the laparotomy.

In Group 3, perfusion deteriorated to a much greater extent than in other groups at 20 hours of observation (MFI, sMFI, PVD, sPVD, PPV and sPPV). There were no significant intergroup differences in the total vessel density (TVD) and small vessel density (sVD) and de Backer score (Table 2).

Throughout the study, the only consistent correlations between microvascular flow and systemic flow were observed in Group 3 (Table 3). Impairment of microvascular flow (expressed by changes in the PPV, sPPV, PVD, sPVD, MFI, sMFI) were related to the heart rate (HR), mean arterial pressure (MAP),diastolic

arterial pressure (DAP), systemic vascular resistance (SVRI) with $p < 0.05$ in all cases. None of these parameters depended on the cardiac output (CO), cardiac index (CI), or pulmonary capillary wedge pressure (PCWP). Nevertheless, they were inversely related to pulmonary pressures – mean pulmonary artery pressure (MPAP), systolic pulmonary artery pressure (SPAP), diastolic artery pressure (DPAP).

Tab 3. Pearson's correlation coefficients for microcirculatory variables, hemodynamic parameters, pH, HCO_3^{2-}, BE, diuresis, creatinine, and urea. For all correlations $p<0.05$ is used.

	PVD	sPVD	PPV	sPPV	MFI	sMFI
MAP	0.70	0.69	0.69	0.70	0.64	0.72
DAP	0.69	0.69	0.69	0.70	0.63	0.72
MPAP	-0.6	-0.59	-0.58	-0.58	-0.5	-0.49
SPAP	-0.48	-0.48	-0.47	-0.47	-0.39	-0.37
DPAP	-0.69	-0.69	-0.67	-0.67	-0.60	-0.60
SVI	0.45	0.44	0.55	0.54	0.58	0.48
SVRI	0.51	0.51	0.49	0.50	0.45	0.54
pH	0.78	0.78	0.76	0.76	0.73	0.74
HCO_3^2	0.79	0.79	0.80	0.80	0.74	0.78
BE	0.80	0.80	0.80	0.80	0.74	0.78
Diuresis	-0.64	-0.65	-0.60	-0.62	-0.56	-0.65
Creatinine	-0.46	-0.47	-0.45	-0.45	-0.46	-0.53
Urea	-0.50	-0.50	-0.47	-0.48	-0.46	-0.56

In terms of the laboratory test results, capillary perfusion deterioration was related to organ dysfunction with a significant decrease of pH and BE, diuresis reduction, increase in creatinine and urea only in Group 3 (Table 3).

Discussion

Flow impairment and heterogeneity are the main pathologies accounting for microcirculatory derangement in sepsis. Endothelium dysregulation, arteriolar constriction, increased leukocyte and platelet adhesion, and heterogeneous expression of inducible nitric oxide synthase cease the flow in some capillaries, reducing functional capillary density [9]. Areas without flow are adjacent to hyperperfused regions. The off-load time for oxygen exceeds the time of erythrocyte passage through capillaries with hyperdynamic flow, which results in the inability of oxygen to detach from hemoglobin

and diffuse [11]. It seems that flow heterogeneity has a worse impact on tissue oxygenation than equally impaired perfusion [2, 27, 28]. The restoration of flow in closed capillaries by NO, a strong vasodilator, might improve tissue oxygenation and elimination of anaerobic metabolism products. Total nitric oxide production in sepsis is increased but its distribution is very heterogenous mainly because of uneven expression of inducible NO synthase [12, 22,23, 29,30]. This creates areas with NO deficiency that might benefit from exogenous NO delivery [21, 23]. The effect of iNO on microcirculation remains unclear [23, 31]. In our study, iNO potentiated by intravenous hydrocortisone had no effect on the proportion of perfused vessels, perfused capillary density, and the microcirculatory flow index. Unfortunately, our study had certain limitations. The studied groups were not numerous, which made statistical analysis impossible for some variables including the heterogeneity index of microvascular perfusion, hemodynamic and laboratory data. Moreover, for technical reasons, lactates were not monitored during the study. Even though functional capillary density was not affected by iNO with hydrocortisone, we are unable to evaluate changes in the heterogeneity of perfusion, lactate washing, or organ function.

Microcirculatory variables changed more profoundly in piglets not resuscitated with fluids and vasopressors, with a statistically greater decrease of the MFI, sMFI, PPV, sPPV, PVD and sPVD. Only in this group was capillary flow strongly related to systemic flow. In groups receiving fluids and noradrenaline, hemodynamic variables were stabilized but these changes did not lead to microcirculatory flow normalization. This observation supports the theory that resuscitation of systemic flow does not resuscitate microcirculation to the same degree as systemic circulation. Capillary flow impairment still remains, but it proceeds more slowly. The discrepancy between hemodynamics and microcirculatory variables has already been described by other authors [3, 5, 32]. According to De Backer et al., microcirculatory derangement may precede systemic hemodynamic compromise and the relation between hemodynamic and microcirculatory indices may be loose [5]. Still, changes in cardiac output and mean arterial pressure may influence capillary perfusion [5]. In a single-center prospective observational study, de Backer proved that when sepsis develops at early stages of resuscitation, hemodynamic measurements are related to microvascular flow indices, and this correlation disappears at later stages [3]. In the human subject research, our observations are limited to septic and resuscitated groups, and are usually compared to healthy control groups. For obvious reasons, a control non-resuscitated group cannot be permitted. It is possible that for this reason no association between systemic hemodynamic and capillary flow indices was observed at advanced stages of sepsis. There is no doubt that hemodynamic resuscitation in sepsis is beneficial [33]. In recent years, we have been seeking correlations between macro- and microcirculatory indices in sepsis. This correlation exists when sepsis proceeds undisturbed. Perhaps the search for a direct relationship between the indicators of micro- and microcirculation and the expectation of microcirculation normalization as soon as global hemodynamics improvement is reached are some erroneous assumptions at an early stage of the therapeutic process and treatment optimization. The fact that microcirculation and systemic circulation are not coherent in resuscitated patients does not mean that hemodynamic resuscitation is not effective in terms of capillary perfusion resuscitation. As shown above, microcirculation deteriorated in resuscitated groups, but more slowly. This suggests that resuscitation may buy time necessary for the immune system and antibiotics

to overcome the infection. This observation suggests that the management of sepsis should first concentrate on the monitoring and stabilization of systemic circulation and – at later stages – on the evaluation and resuscitation of microcirculation [5].

Moreover, in the non-resuscitated group, microcirculatory parameters were significantly related to the progress of acidosis and renal failure. This correlation was not observed in resuscitated groups, where acidosis and organ dysfunction did not proceed as fast in the non-treated group. In Group 3, the combination of capillary perfusion deterioration and related metabolic acidosis supports the theory of microcirculatory pathogenesis of anaerobic metabolism.

In our study, the TVD, sVD and de Backer score did not follow any certain pattern and their changes over time were not statistically significant. This observation does not support the results presented by Massey from the ProCESS trial [34]. In the above study, the TVD and de Backer score appeared to be strongly associated with outcome in septic patients. However, their prognostic value appeared to be significant after 72 hours of observation. The observed difference in the TVD and de Backer score value might result from a shorter time of observation and a smaller group of animals in our study.

Our study had certain limitations. The number of animal subjects in the study groups was limited and the statistical analysis of some parameters was not possible.

Finally, our observations of microcirculatory alterations were limited to the sublingual area. The sublingual mucosa and the digestive mucosa have the same embryologic origin, and changes in their capnometry correlate quite well, showing similar alterations [21, 35, 36]. Capillary perfusion in the sublingual area seems to reflect the flow in the splanchnic mucosa and is easily accessible. Nevertheless sidestream dark field imaging does not allow direct in vivo observation of microcirculation in vital organs.

Materials And Methods

Ethical Issues

The study was approved by the Animal Research Ethics Committee of the Institute of Immunology and Experimental Therapy, Polish Academy of Science, Wroclaw, Poland (permission number 7/05) and reported in compliance with the ARRIVE guidelines. All methods were carried out in accordance with relevant guidelines and regulations. Care provided to animals was compliant with the European Convention for Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [37].

Throughout the research, care was taken to guarantee maximum comfort to animals, which were generally anesthetized, and the depth of anesthesia was adjusted to the level of painful stimulation. Those that survived the study period were euthanized with pentobarbital.

Animal Preparation

A detailed description of the instrumentation was presented elsewhere earlier [38]. This study is part of a large project covering a sequence of studies on iNO delivery to septic piglets.

The experiment was conducted at the Department of Surgery at the Wroclaw University of Environmental and Life Sciences, Poland. Originally, the study group consisted of 12 piglets of Polish breed. One of them was excluded as it developed severe hypotonia unresponsive to treatment during instrumentation and died. All animals were of the same age (2 months) and their median body weight was 18.5 kg (range 17–22 kg).

All piglets fasted during nocturnal hours before the experiment; however, they were allowed to drink water at will. To induce anesthesia, we administered zolazepam/tiletamine 4 $mg \times kg^{-1}$ dissolved in medetomidine 0.08 $mg \times kg^{-1}$ intramuscularly. All animals were successfully intubated and mechanical ventilation was started. The ventilation protocol was based on a pressure-controlled mode and aimed to obtain the inspired fraction of oxygen (FIO_2) of 0.3 and the PEEP of 5 cm H_2O. During the experiment, ventilator settings were adjusted in accordance with blood gas analysis. Recruitment maneuvers and airway suctioning were allowed.

Following induction, general anesthesia was maintained with intravenous infusion of ketamine (1.5–2.4 $mg \times kg^{-1} \times h^{-1}$), medetomidine (5.3–8.2 $\mu g \times kg^{-1} \times h^{-1}$), fentanyl (0.8–1.3 $\mu g \times kg^{-1} \times h^{-1}$), and midazolam (0.08–0.13 $\mu g \times kg^{-1} \times h^{-1}$). All animals received a solution of 2.5% dextrose in 0.9% saline (Glu/NaCl 1:1) at a constant rate of 100 $mL \times h^{-1}$. For further hemodynamic monitoring, arterial lines, central venous catheters, and pulmonary artery catheters were introduced. Access to the urinary bladder was obtained transabdominally. According to the core temperature measurement, heating blankets and external cooling were used to keep the temperature within the normal range.

Study Protocol

The timeline of interventions is presented in Fig. 1. After a one-hour rest, all piglets underwent a surgical midline laparotomy. The descending colon was visualized, a 3-cm incision was performed, and 1.5 g/kg of fecal content were removed, mixed with blood and then deposited close to the diaphragm, in order to cause a septic-like condition. Then, the laparotomy was closed.

Next, the piglets were randomly allocated to one of three groups:

1. Group 1 - piglets receiving iNO (Pulmonox-Messer Griesheim 800 ppm iNO in 9000 nitrogen) at a concentration of 30 ppm and hydrocortisone (75 mg intravenously every 7 hrs). Hypotensive piglets (MAP < 60 mmHg for more than 3 minutes) were treated with a bolus of Ringer's lactate (300 ml) and norepinephrine infusion (if unresponsive to fluids) to maintain MAP > = 65.
2. Group 2 - piglets not receiving iNO or hydrocortisone but treated for hypotension with fluids and noradrenaline infusion as in the case of Group 1;
3. Group 3 - piglets not receiving iNO and hydrocortisone, not resuscitated with fluids or noradrenaline infusion

Microcirculation Assessment

The study evaluated microcirculation with the MicroScan™ device (MicroVisionMedical, Amsterdam, the Netherlands) based on sidestream dark field imaging (SDF). We assessed the microvascular bed in the sublingual region of the oral cavity in four different sequences at every time point. Every record was stored by a random number code and the analyzing researcher was blinded to all clinical data. To optimize image quality and avoid artefacts, we ensured maximum camera stability, removed saliva from the sublingual area using sterile gauze, and minimized pressure on the mucosa.

For the microcirculation evaluation, we used AVA 3.0, which automatically identifies vessels. It calculates total vessel density, the number of vessels crossing a grid of six lines (dividing the screen image into 16 equal areas), and the de De Backer score (total number of vessel crossings per grid length). Further images were assessed in two semi-quantitative methods described elsewhere by Boerma and de Backer [3, 6]. The analysis provided information about the proportion of perfused vessels (PPV), perfused vessel density (PVD = PPVxTVD), and microvascular flow index (MFI). All the above variables were separately calculated for small vessels whose diameter was smaller than 20 μm (small vessel MFI-sMFI, small vessel density-sVD, proportion of perfused small vessels-sPPV, perfused small vessel density-sPVD). We also calculated the heterogeneity index (HI), which was proposed by Trzeciak et al. for microvascular evaluation [3].

Statistical Analysis

The data were analyzed using STATISTICA ver. 12. A one-way ANOVA was performed for a comparative analysis of microcirculatory variables obtained for the studied groups at each time point. Pearson's coefficients were used to assess the correlation between microcirculatory indices and hemodynamic variables, and between microcirculatory parameters and laboratory data. A p-value of < 0.05 was considered significant. All values are reported as medians, unless otherwise stated.

Declarations

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

MG performed the experiments, acquisited, analyzed and interpreted the data, wrote the manuscript.

WG designed the study, supervised this study, performed the experiments, analyzed and interpreted the data.

PH performed the experiments, acquisited and analyzed the data.

CF designed the study, supervised this study, performed the experiments.

All of the authors have read, revised. and approved the submitted version of the manuscript.

COMPETING INTERESTS
The author(s) declare no competing interests.

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Figures

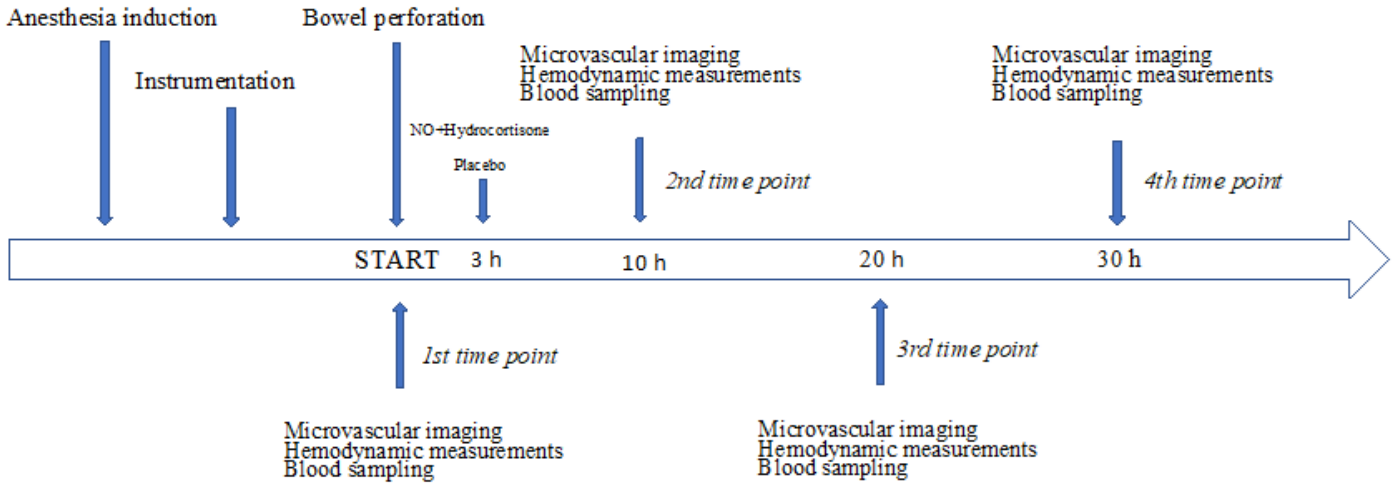

Figure 1

Timeline of intervention and data acquisition events.

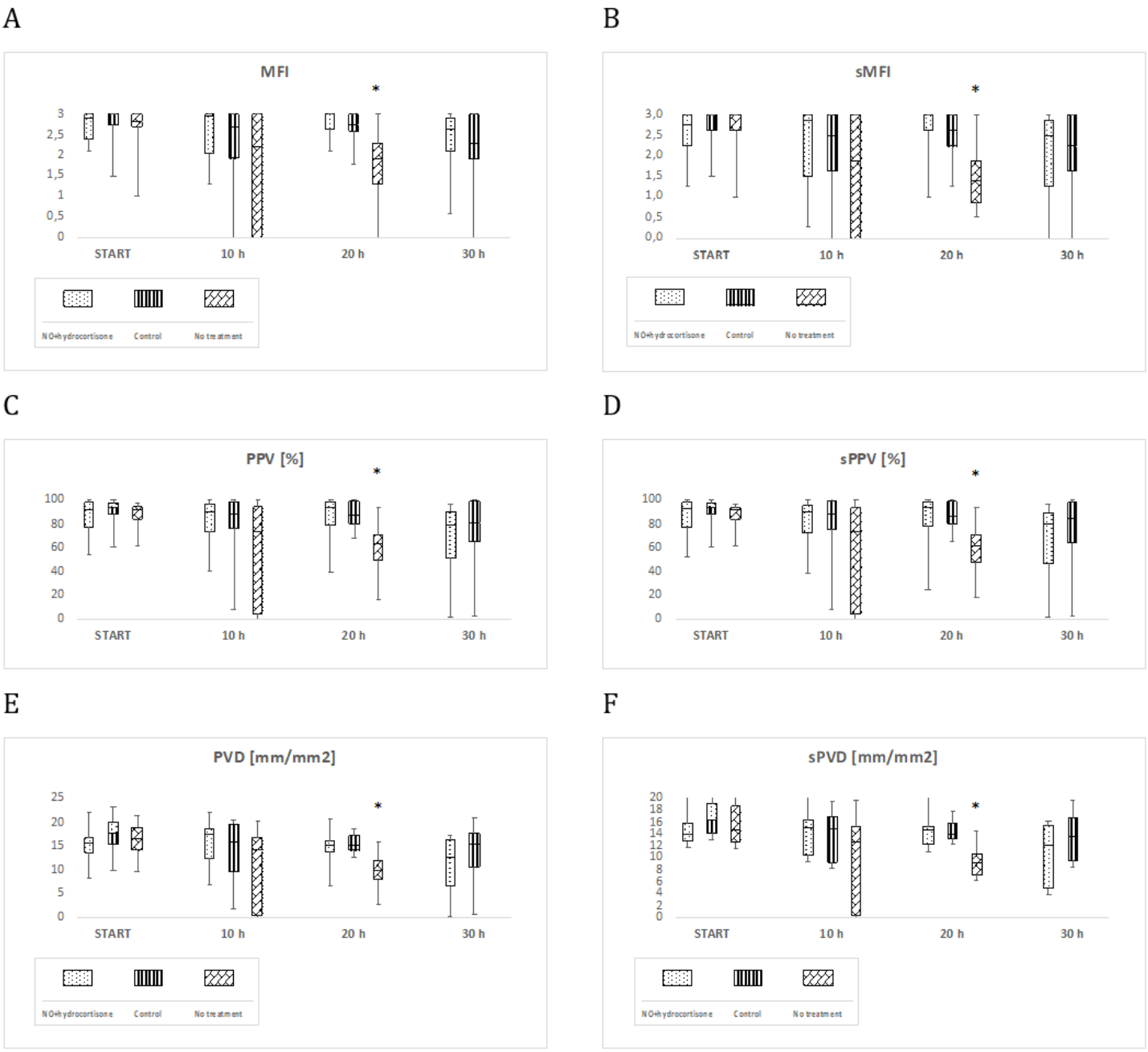

Figure 2

Baseline microcirculatory variables and their time course in the studied groups. Initially, there were no differences in microcirculatory variables between the groups. At 20 hours of observation, a significant difference was seen between the groups, with a marked decrease of the MFI (A), sMFI (B), PPV (C), sPPV (D), PVD (E), and sPVD (F) in the group without treatment (p ranging from 0.01 to 0.04). The piglets from this group died before the final point (30 hours). The analysis of the survivors from the group receiving iNO + hydrocortisone and the control group showed no difference in the MFI, sMFI, PPV, sPPV, PVD, and sPVD between these groups in the time course. Microcirculatory variables are presented as medians. The data are presented as medians (25th–75th percentiles).