Effects of hyperoxia on local and remote microcirculatory inflammatory response after splanchnic ischemia and reperfusion

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Waisman, Dan, Vera Brod, Rafael Wolff, Edmond Sabo, Mark Chernin, Zalman Weintraub, Avi Rotschild, and Haim Bitterman. Effects of hyperoxia on local and remote microcirculatory inflammatory response after splanchnic ischemia and reperfusion. Am J Physiol Heart Circ Physiol 285: H643–H652, 2003. First published April 24, 2003; 10.1152/ajpheart.00900.2002.—Splanchnic ischemia-reperfusion (I/R) causes tissue hypoxia that triggers local and systemic microcirculatory inflammatory responses. We evaluated the effects of hyperoxia in I/R induced by 40-min superior mesenteric artery (SMA) occlusion and 120-min reperfusion in four groups of rats: 1) control (anesthesia only), 2) sham operated (all surgical procedures without vascular occlusion; air ventilation), 3) SMA I/R and air, d) SMA I/R and 100% oxygen ventilation started 10 min before reperfusion. Leukocyte rolling and adhesion in mesenteric microvessels, pulmonary microvascular blood flow velocity (BFV), and macromolecular (FITC-albumin) flux into lungs were monitored by intravital videomicroscopy. We also determined pulmonary leukocyte infiltration. SMA I/R caused marked decreases in mean arterial blood pressure (MABP) and blood flow to the splanchic and hindquarters vascular beds and pulmonary BFV and shear rates, followed by extensive increase in leukocyte rolling and adhesion and plugging of >50% of the mesenteric microvasculature. SMA I/R also caused marked increase in pulmonary sequestration of leukocytes and macromolecular leak with concomitant decrease in circulating leukocytes. Inhalation of 100% oxygen maintained MABP at significantly higher values (P < 0.001) but did not change regional blood flows. Oxygen therapy attenuated the increase in mesenteric leukocyte rolling and adherence (P < 0.0001) and maintained microvascular patency at values not significantly different from sham-operated animals. Hyperoxia also attenuated the decrease in pulmonary capillary BFV and shear rates, reduced leukocyte infiltration in the lungs (P < 0.001), and prevented the increase in pulmonary macromolecular leak (P < 0.001), maintaining it at values not different from sham-operated animals. The data suggest that beneficial effects of normobaric hyperoxia in splanchic I/R are mediated by attenuation of both local and remote inflammatory microvascular responses.

reperfusion injury; acute lung injury; reoxygenation injury; intravital videomicroscopy; multiorgan failure; systemic inflammatory response syndrome

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HYPOPERFUSION AND ISCHEMIA of the bowel is a common, important pathological process that affects adults and infants. In both groups the morbidity and mortality related to these processes are high. In adults, occlusive and nonocclusive conditions may reduce splanchic blood flow (8, 35). Occlusion of a large vessel may be a consequence of emboli, thrombosis, tumors, aneurysm, or surgical procedures (i.e., aortic surgical repair). In small vessels occlusion may be the result of vasculitis or thrombosis. Causes of nonocclusive splanchic ischemia in adults are major loss of blood, septic shock, severe cardiac valvular disease, cardiomyopathies, and heart failure (8). In newborn infants, necrotizing enterocolitis is a syndrome of acute ischemic intestinal necrosis of unclear, probably multifactorial, etiology. It occurs in 3–5% of all neonatal intensive care unit admissions and is characterized by long-term morbidity and high mortality (17).

Oclusive and nonocclusive pathologies both result in tissue ischemia, microbial proliferation, and translocation of bacteria or their toxic products that activate a variety of inflammatory mediators and chemotrac- tants. These mediators originate from the affected hypoperfused-hypoxic cells and also from local and circulating white blood cells (WBC) and macrophages (9).

During splanchic ischemia, cytokines (TNF-α, IL-1, and IL-8), platelet-activating factor (PAF), eicosanoids, leukotrienes, reactive oxygen species, the complement system, and activated adhesion molecules on leukocytes, macrophages, and endothelial cells are all involved in the activation of local and remote inflammatory cascades that culminate in a systemic inflammatory response (SIR). Reperfusion of the ischemic organs may even aggravate the inflammatory process, at least temporarily (9, 12).

As a consequence of the SIR provoked by the primary pathology in the bowel many organs are affected, producing multiple-system organ failure manifested as severe hypotension, heart failure, oliguria and renal failure, stupor or coma, and respiratory failure. The
remote effects of splanchnic ischemia on pulmonary function are responsible for further deterioration and the need for mechanical ventilatory support to sustain life (11).

We studied the effects of inhalation of 100% oxygen on regional and systemic hemodynamics and used real-time intravital videomicroscopy to monitor mesenteric and pulmonary microcirculatory inflammatory changes during splanchnic ischemia-reperfusion (I/R).

MATERIALS AND METHODS

Animal preparation. Male Sprague-Dawley rats weighing 180–230 g were used for the experiments. Housing included day and night cycles with temperature control. Standard chow and water were administered ad libitum. The experimental protocol received the approval of The Committee for the Supervision of Animal Experiments, Technion-Israel Institute of Technology.

The rats were anesthetized with Inactin (100 mg/kg ip; RBI, Natick, MA) and tracheostomized with a polyethylene cannula (PE-240). The right carotid artery was cannulated with polyethylene tubing (PE-50) for monitoring systolic, diastolic, and mean blood pressure and heart rate (DigiMed Blood Pressure Analyzer; Micro-Med, Louisville, KY) and for withdrawal of blood. Hemodynamic data were collected, displayed, and stored with Digi-Med System Integrator DMSI-200/4 (Micro-Med). The animals’ temperature was monitored continuously with a rectal probe and a Temperature Monitor TH-8 (Physitemp Instruments, Clifton, NJ) and was maintained at physiological limits (36.0–37.5°C) with a heating pad.

After a midline laparotomy, the superior mesenteric artery (SMA) was isolated near its aortic origin and looped with a 1-0 silk suture. The silk suture was then threaded through a single piece of PE-240 tubing with one end flame blunted. This piece was advanced to the SMA without occluding it. Once the abdominal incision was closed, the SMA could be occluded or reperfused by movement of the tube without reopening the incision. During the period of ischemia the incision in the abdominal wall was kept approximated to prevent fluid and heat loss. After a 40-min period of ischemia the tubing was released, thus starting the reperfusion period. After completion of all surgical procedures, animals were allowed a 30-min stabilization period before the beginning of the experiments.

Regional hemodynamic studies. An ultrasonic blood flow probe (Transonic System, Ithaca, NY) was placed on the SMA near its aortic origin or on the distal portion of the descending aorta near the iliac bifurcation for continuous measurement of regional blood flow to the small bowel and hindquarters. Blood flow at the intestinal serosa was monitored with a laser-Doppler flowmeter (Advance Laser Flowmeter ALP21; Advance, Tokyo, Japan).

The effects of exposure to hyperoxia on mean arterial blood pressure (MABP) and regional blood flows were determined at all stages of the experimental protocol. The experimental information was displayed and analyzed on- or off-line with data acquisition and analysis hardware and software (Windaq; Dataq Instruments, Akron, OH). Vascular resistance was calculated as MBAP divided by the mean respective regional blood flow (in the SMA or distal aorta; expressed as mmHg·mL⁻¹·min⁻¹).

Videomicroscopy. All observations were done with a Olympus trinocular microscope BX-60 (Tokyo, Japan) equipped with epi-illumination provided by a 100-W mercury lamp. Two sets of filters were used: one neutral, to decrease heating and damage to the lung tissue, and the second, 420–480 nm (U-MSWB), to achieve the green fluorescent spectrum. Experiments were recorded with a Sony 3CCD color video camera coupled to the microscope with a DXC-950P camera adaptor (CMA-D2). The camera was connected to a Sony digital videocassette recorder (DVCAM DSR-30P) and in parallel to a high-resolution Sony Trinitron color video monitor (PVM-20M4E).

The images obtained were processed off-line with a PC connected to the videocassette recorder. Image processing was performed with a still-image capture board (DVBK 2000; Sony). Morphometric studies and fluorescence intensity measurements were performed with an image analysis software program (Image-Pro Plus; Media Cybernetics).

Preparation for videomicroscopy of mesenteric microcirculation. A terminal section of intestinal mesentery was exteriorized through a midline abdominal incision, draped over a transparent pedestal, and covered with a nylon wrap. The temperature of the intestinal loop was kept constant at 37°C with a microcomputer-based temperature controller (PCS-100; Shinko, Tokyo, Japan). A fragment of the microvascular bed was mapped, and postcapillary venules were selected for observation. The same vessels were studied in all stages of the experiment. Final magnification on the screen was ×1,000.

All measurements were done off-line. Vessel diameter was measured with a ruler. Leukocytes were considered to be rolling if they were moving at a velocity slower than that of erythrocytes. Rolling was expressed as the number of cells moving past a designated point over 30 s. Adherence was defined as the number of leukocytes that remain stationary for 30 s and is reported as the mean number of leukocytes per 50-μm vessel length. All videomicroscopic measurements were performed in the following time periods: baseline (before SMA occlusion and reperfusion), 30, 60, and 90 min after start of reperfusion). Microvessel patency was determined by the presence or absence of blood flow (plugged vessel).

Preparation for videomicroscopy of lung subpleural microcirculation. After the laparotomy, mechanical ventilation was started. A pressure-cycled infant ventilator (Bourns BP 200 Infant Pressure Ventilator; Riverside, CA) was used to provide mechanical ventilatory support. Initial settings were fraction of inspired O₂ 0.21, peak inspiratory pressure 12 cmH₂O, positive end-expiratory pressure 1 cmH₂O, respiratory rate 50, inspiratory time 0.5 s, and inspirium-to-expirium ratio 1:1.5. With these settings, initial arterial blood gases could be maintained within physiological limits when a steady state was achieved after surgery, i.e., pH >7.3 and arterial PICO₂ = 35–40 mmHg.

The animal was placed in a left lateral decubitus position, a right thoracotomy was performed and the ribs were kept separated with a colibri retractor (Fine Science Tools, North Vancouver, BC, Canada). The lower part of the right upper lobe, the middle lobe, and the upper part of the right lower lobe were exposed by this procedure. The exposed area was covered with a thin plastic wrap to minimize exposure and evaporative fluid losses. The coverage was replaced as needed if fluid or air was present under the nylon film. The lung surface was flushed with warmed normal saline (37°C) during this procedure.

Macromolecular leak. After completion of the surgical preparation, the animals were given an intra-arterial injection of 50 mg/kg FITC-conjugated albumin (FITC-albumin; Sigma, St. Louis, MO) and were allowed a 30-min stabilization period before the start of videomicroscopic observation.
Videomicroscopic images of the lung surface were obtained before, during, and after ischemia of the bowel at 30-min intervals for up to 120 min after reperfusion or until MABP persistently fell below 40 mmHg.

Images from three different areas of the lung subpleural surface were chosen from each observation period for off-line analysis. The images were obtained during the inspiratory phase of the respiratory cycle without any changes in the ventilator setup. The final magnification used was ×500. Fluorescence intensity at the lung surface area was measured in three consecutive frames at each preestablished time point during the study. The mean values for fluorescence intensity of the whole frame were obtained, and the mean value of three frames obtained at each point was calculated.

**Capillary blood flow velocity.** Measurement of capillary blood flow velocity (BFV) at the lung surface microcirculation was performed at baseline (before SMA occlusion) and 60 and 90 min after reperfusion. Observations were started after injection of 0.3 ml of rhodamine G6 (200 μg/ml), which allowed identification of the lung surface vessels during fluorometric microscopy. After this first step, green fluorescent microspheres (diameter 1 μm; Molecular Probes) were injected into the systemic circulation through the arterial catheter at a rate of 0.1 ml/min by an infusion pump. The microspheres were injected as a 20× diluted suspension in normal saline and 5% BSA to prevent adhesion and to increase their circulation time. The injected amount depended only on their time of appearance on the monitor screen during the procedure. When a clear view of the circulating microspheres was obtained, the image was recorded on different areas of the lung surface. Calculations of BFV were performed off-line by frame-by-frame screen measurements of the distance covered by individual microspheres per unit of time. Because the video camera takes a frame every 0.04 s (1/25), velocity was calculated by using the formula $V = \frac{D}{8 \times (velocity/diameter)}$ (18, 22, 30).

**Blood counts.** Cell counts were performed in whole blood obtained from the carotid artery catheter to follow changes in the hematocrit and WBC counts. The samples were obtained at baseline and at the end of the experiments. Hematocrit was measured after microcentrifugation (International Equipment, Boston, MA). Cell counts were performed by Coulter analysis (Bayer Advia 120; Diamond Diagnostics, Holliston, MA).

**Immunohistochemistry for lung tissue leukocyte identification and count.** After completion of the experimental protocol, the rat received an intravenous pentobarbital sodium overdose and the lungs were then excised. The right lung was inflated with formalin with a standardized system providing the rat received an intravenous pentobarbital sodium overdose in a microwave oven. Rabbit anti-human myeloperoxidase polyclonal antibody was used (1:1,000, AO398; Dako, Glostrup, Denmark). The immune complex was marked with avidin-biotin-peroxidase for visualization. The sections were counterstained with hematoxylin to visualize the background tissue.

After sections were stained, the number of leukocytes was counted and divided by the surface of solid lung tissue from each frame and an index of the number of leukocytes per tissue area was obtained (leukocytes/mm²). Tissue area measurements were made with graphic analysis software (Image-Pro Plus, Media Cybernetics).

**Group assignment.** The animals were randomly assigned to one of the following four experimental groups: 1) control rats that did not undergo any surgical procedure were anesthetized and killed, and their lungs were used for baseline morphometric comparative studies; 2) sham-operated rats that underwent all the surgical procedures without occlusion and reperfusion of the SMA were ventilated with room air; 3) rats that underwent SMA I/R received ventilation with air; 4) rats that underwent SMA I/R received ventilation with 100% oxygen starting 10 min before reperfusion and continuing until the end of the experiment.

Because of the extensive surgical procedures used in this study, videomicroscopic imaging of the mesentery or the lung was done in separate groups of animals. Thus, in each experimental animal, only one vascular videomicroscopic technique was performed. Eight to ten animals were studied in each group.

**Statistical analysis.** Values are presented as means ± SE. Differences within groups were evaluated by repeated-measures ANOVA. Differences between specific data means of different groups were evaluated by Tukey’s test.

**RESULTS**

**Hemodynamics.** Figure 1 illustrates the time course of MABP in the three experimental groups of splanchnic I/R and sham-operated rats. MABP at the beginning of the experiment and just before the vascular
occlusion was comparable in the three groups. A slight, statistically insignificant decrease in MABP was found in the sham-operated group thereafter. Occlusion of the SMA caused a comparable increase in MABP ($P < 0.05$). Inhalation of oxygen induced a further significant increase in MABP ($P < 0.03$). Reperfusion of the SMA was followed by an abrupt fall in MABP that reached 90 mmHg in the untreated group. In contrast, in oxygen-treated rats, MABP was maintained at significantly higher values throughout the rest of the experimental protocol ($P < 0.001$ from the untreated group).

Figure 2 demonstrates vascular resistance in the hindquarters (Fig. 2A) and blood flow to the distal aorta (Fig. 2B) in sham-operated and I/R rats. Values are means ± SE. *Hindquarter vascular resistance in I/R + oxygen group significantly higher than before inhalation ($P < 0.01$).

Figure 3 displays resistance in the SMA vascular bed (Fig. 3A), blood flow in the SMA (Fig. 3B), and tissue perfusion of the small bowel serosa (Fig. 3C) in the three experimental groups. SMA vascular resistance increased gradually after reperfusion in the two I/R groups ($P < 0.05$). Oxygen treatment did not cause a significant change in any of the regional splanchnic hemodynamic parameters.

Mean arterial blood $PO_2$ in the SMA I/R group ventilated with air was $91 ± 4$ mmHg. In the SMA I/R group ventilated with 100% oxygen, mean arterial blood $PO_2$ increased to $472 ± 17$ mmHg ($P < 0.0001$).

Mesenteric microcirculation. Figure 4 illustrates rolling of leukocytes in mesenteric venules (Fig. 4A), leukocyte adhesion (Fig. 4B), and the percentage of plugged mesenteric microvessels (Fig. 4C). No signifi-

Fig. 2. Hindquarter vascular resistance (A) and blood flow to the distal aorta (B) in sham-operated and I/R rats. Values are means ± SE. *Hindquarter vascular resistance in I/R + oxygen group significantly higher than before inhalation ($P < 0.01$).

Fig. 3. Vascular resistance in the superior mesenteric artery (SMA) bed (A), blood flow to the SMA (B), and perfusion of the small bowel (C) in I/R and sham-operated rats. Values are means ± SE.
A significant change was observed in the number of rolling leukocytes in the sham-operated group throughout the experiment. Splanchnic I/R caused a gradual increase in leukocyte rolling in untreated rats, reaching a value fourfold higher than the initial control value of the group 60 min after reperfusion (P < 0.0001). In contrast, inhalation of oxygen prevented the increase in rolling. The final rolling values in the oxygen-treated group were significantly lower than those of the untreated I/R group (P < 0.0001) and not significantly different from those in sham-operated rats.

Splanchnic I/R caused a gradual increase in microvascular plugging (Fig. 4C), reaching almost 60% at final stages of the experiments (P < 0.05). Oxygen treatment almost totally abolished this phenomenon (P < 0.05 from untreated rats) without significant difference from sham-operated rats.

Lung macromolecular leak. Figure 5 shows typical sequential fluorescent images of the lung surface after a single intra-arterial injection of FITC-albumin in a rat subjected to splanchnic I/R. This series of videomicroscopic reproductions of the lung at the different stages of the experiment demonstrates the gradual increase in the intensity of green fluorescence in both interstitial and alveolar spaces. Figure 6 summarizes percent changes in lung area fluorescence intensity in the three experimental groups. No significant change occurred in the sham-operated group throughout the experiment. In untreated I/R rats a gradual increase was detected, reaching an average 162 ± 22% increase in area fluorescence during late stages of reperfusion (P < 0.001). This increase was almost totally prevented by inhalation of oxygen (P < 0.001 vs. untreated group and no significant difference from sham-operated group).

Capillary BFV and wall shear rate. BFV was measured at the capillary level in the subpleural microcirculation by direct observation of circulating fluorescent microspheres. Velocity and shear rates were calculated on the basis of these observations. Baseline capillary BFV values were not significantly different among the three experimental groups and ranged from 732 to 887 μm/s. Figure 7 illustrates changes in capillary BFV in the three experimental groups. No significant differences were detected in the sham-operated group. In contrast, the I/R plus air group demonstrated 36 ± 3% and 46 ± 2% decrease in capillary BFV 60 and 90 min after reperfusion, respectively. This decrease was significantly attenuated by oxygen therapy.

Baseline capillary shear rates were not significantly different among the three experimental groups and ranged from 732 to 887 s⁻¹. Figure 8 summarizes changes in capillary shear rates in the three experimental groups. No significant differences were detected in the sham-operated group. I/R caused a marked drop of 36 ± 3% and 46 ± 2% in capillary shear rates 60 and 90 min after reperfusion, respectively. This decrease was significantly attenuated by oxygen therapy.

Lung morphometry. Figure 9 shows the number of leukocytes per lung solid tissue area (Fig. 9A) and the percentage of lung solid tissue area covered by leukocytes (Fig. 9B). As demonstrated in Fig. 9A, untreated I/R rats exhibited a more than threefold increase in leukocyte sequestration in the lung (P < 0.01 vs. controls and P < 0.05 vs. sham-operated rats). Oxygen therapy started 10 min before reperfusion significantly attenuated the increase in leukocyte infiltration (P <
0.001). Similar findings are illustrated in Fig. 9B, which shows a fourfold increase in lung solid tissue area covered by leukocytes and a significant attenuation of this increase in oxygen-treated rats to values not significantly different from those in the sham-operated group.

Blood counts. Baseline hematocrits ranged between 46% and 48%. The hematocrit did not change significantly in the sham-operated group. In contrast, a significant, similar increase in the hematocrit occurred in the two I/R groups, reaching 58 ± 3% and 59 ± 2% in the air- and oxygen-treated groups, respectively.

Figure 10 summarizes initial and final WBC counts in the three experimental groups. Splanchnic I/R caused a significant decrease in WBC count. Oxygen therapy maintained WBC count at a value that was significantly higher than that in the air-treated group.
DISCUSSION

In this study we evaluated the effects of inhalation of 100% oxygen on local and remote consequences of splanchic I/R. Inhalation of oxygen increased blood pressure and maintained it at significantly higher values than in untreated animals throughout the experimental protocol. Oxygen induced vasoconstriction in the hindquarters without a significant change in absolute values of blood flow to that region. Inhalation of oxygen did not change splanchic hemodynamic variables (SMA resistance, SMA flow, and perfusion of small bowel serosa). Oxygen therapy exerted major beneficial effects on the local splanchic microcirculation. It attenuated leukocyte rolling and adhesion to the mesenteric endothelial surfaces and maintained the patency of mesenteric microvessels. Furthermore, oxygen decreased the sequestration of leukocytes in the lungs and markedly attenuated the increase in pulmonary macromolecular leak that followed splanchic I/R.

Splanchnic ischemia is a common clinical entity that may be caused by mechanical obliteration of flow in blood vessels (occlusive ischemia) or by critical secondary reduction in splanchic organ blood flow (nonocclusive ischemia) (8, 11, 14, 29, 35). Occlusion of a large vessel (e.g., the SMA) may be due to emboli, thrombosis, or pressure by a tumor, an aneurysm, or surgical ligation. Thrombosis or vasculitis may occlude smaller vessels. Nonocclusive ischemia is a consequence of low-flow states in which there is no vascular occlusion or in which the degree of vascular narrowing (e.g., due to atherosclerosis) is not enough by itself to account for the failure of perfusion. Nonocclusive ischemia usually results from a local circulatory response during states of globally reduced oxygen availability due to hypovolemia, hypotension, and/or reduced cardiac output (8, 11, 14).

The splanchic region is rapidly injured by both occlusive and nonocclusive ischemia because of the deprivation of vital nutrients, particularly oxygen. The resulting cellular hypoxia activates a variety of vascular and inflammatory mediator systems (e.g., eicosanoids, leukotrienes, PAF, reactive oxygen species, the complement system, cytokines, and adhesion molecules) that trigger adhesion, migration, and activation...
of WBC and increase vascular leakage with fluid and protein efflux from blood vessels culminating in severe local inflammatory response and SIR (4, 11, 21, 29, 39). Although restitution of blood flow to ischemic tissues is critical to their salvage, the reperfusion of ischemic splanchnic organs may paradoxically enhance the vascular and tissue inflammatory response initiated by the ischemia and even aggravate (at least temporarily) some of the manifestations of the ischemic damage (4, 21, 29, 39).

In light of the key importance of tissue hypoxia in the initiation and propagation of local and systemic inflammatory responses during splanchnic I/R, it is rational to evaluate the effects of administration of oxygen at high ambient partial pressures in this condition. In a previous study we demonstrated a protective effect of hyperbaric oxygen after splanchic I/R. Postreperfusion treatment with oxygen maintained MABP, decreased plasma accumulation of lysosomal enzyme cathepsin D activity and increase in hematocrit, and resulted in better survival (4, 5).

Administration of oxygen at high partial pressures augments oxygen delivery and may alleviate tissue hypoxia. However, oxygen availability to the tissues is also determined by hemodynamic variables (e.g., peripheral resistance and regional blood flows). It has long been established in healthy animals and humans that oxygen causes a temporary increase in blood pressure by increasing total peripheral vascular resistance secondary to generalized peripheral vasoconstriction (15, 19, 27). This transient change is rapidly counter-balanced in healthy subjects by a decrease in heart rate and cardiac output that prevents a sustained effect on arterial blood pressure (19). Only limited information is currently available on generalized and regional hemodynamic and microvascular effects of hyperoxia in models of ischemia and shock. In previous studies done in hemorrhagic shock (1, 6, 7) and in splanchic I/R shock (4), we showed that hyperoxia induces a marked, sustained increase in MABP. In hemorrhagic shock, this was accompanied by a significant increase in vascular resistance in the hindquarters and a concomitant decrease in blood flow in the distal aorta and skeletal muscle (6). Furthermore, oxygen did not change vascular resistance in the renal and splanchnic beds and induced a significant increase in blood flow to the renal artery, SMA, and small bowel (6). It was suggested that redistribution and shifting of blood flow to the splanchnic and renal vascular beds could be a major mechanism of the beneficial effects of hyperoxia in hemorrhagic shock that resulted in improved short- and long-term survival (1, 7). In the present study oxygen exerted a similar pattern of hemodynamic effects. It increased blood pressure and hindquarters vascular resistance and had no effect on SMA vascular resistance. However, in contrast to our findings in hemorrhagic shock, the similar differential regional effect of oxygen on vascular resistance was not followed by significant differences between treated and untreated animals in blood flows in the main vascular conduits to the hindquarters (distal aorta) and small bowel (SMA). Thus a shift in blood flow to the splanchnic vascular bed was not found in this model of splanchnic I/R.

As expected, in our model, I/R induced marked gradual increase in leukocyte rolling and adhesion to the mesenteric microvascular endothelium and a concomitant decrease in peripheral blood WBC counts. This was followed by plugging of 50–60% of the mesenteric microvessels toward the end of the experimental protocol. The major role of leukocyte-endothelial cell adhesion in the pathophysiology of I/R injury is well established (12, 28). Endothelial adhesion, as well as activation and transmigration of leukocytes across endothelial surfaces to the tissues, leads to microvascular dysfunction and local and systemic inflammatory responses (11, 28). The recruitment and adhesion of leukocytes to endothelial surfaces is also one of the mechanisms of postischemia blockade of microvascular flow, commonly termed the “capillary no-reflow phenomenon,” that hampers full reinstitution of microvascular blood flow after reperfusion and is a most important mechanism of the irreversibility of tissue damage after significant periods of ischemia (12).

In our experiments, inhalation of 100% oxygen at normal atmospheric pressure prevented the increase in both rolling and adhesion of leukocytes in mesenteric microvessels and thus diminished the local inflammatory response to ischemia and reperfusion. Oxygen therapy prevented the decrease in peripheral blood WBC count and maintained it at values that were not significantly different from baseline counts in this group. Hence, the beneficial effects of oxygen on rolling and adhesion of leukocytes to microvascular endothelium are not caused by lowering of WBC counts in peripheral blood. It is suggested that the maintenance of WBC counts by oxygen is secondary to its effect on attenuation of leukocyte adhesion to microvascular endothelium. It is also suggested that the decrease in rolling in the I/R plus air group toward the end of the experiments may be explained by the extensive adhesion of WBC that reached a maximum at this time point.

The commonly accepted paradigm of I/R injury emphasizes the role of oxygen-derived free radical formation in activation of the inflammatory cascade by formation of inflammatory mediators and nuclear transcription factors, upregulation of adhesion molecules, and consequently, an increase in leukocyte-endothelial cell adhesion and microvascular barrier disruption (12, 28). This understanding of the central role of reactive oxygen species in I/R injury evoked an initial concern that hyperoxia could exacerbate the injury by adding extra oxygen to the system and thus increasing free radical formation (3, 10). However, a significant body of experimental data from biochemical studies and from animal models of I/R in skin flaps and skeletal muscle supports a protective role of hyperoxia by blocking neutrophil-endothelial adherence and inhibiting subsequent arteriolar vasoconstriction (16, 32, 37, 38). Furthermore, a generalized anti-inflammatory action of hyperbaric oxygen (HBO) has also been demon-
strated in an animal model of zymosan-induced SIR and shock (24). In this regard, it has been shown in ischemic skin flaps treated with HBO that hyperoxia increases local endothelial surface SOD activity (16). This action of hyperoxia may diminish the more distal proinflammatory events initiated by oxygen-derived free radicals after I/R. Moreover, it has been demonstrated that HBO inhibits neutrophil β2-integrin expression (2). However, this observation was recently challenged by a study in which neutrophil CD18 expression after I/R of the skeletal muscle was not changed by HBO (20). Together, these observations suggest a direct local and systemic action of hyperbaric hyperoxia at the neutrophil-endothelial interface that may underlie its local and systemic anti-inflammatory actions after I/R. The full extent of the cellular mechanisms of the protective effects of hyperoxia in I/R injury awaits clarification. We are unaware of studies on cellular and systemic in vivo effects of normobaric hyperoxia in models of I/R. In our experiments splanchnic I/R caused a marked decrease in MABP, transient cessation of blood flow in the SMA, and significant reduction in pulmonary microvascular blood flow velocity and shear rate. Normobaric hyperoxia induced a sustained increase in MABP. However, this change in vascular pressure did not yield a significant effect on SMA flow. In contrast to the lack of effect on splanchnic macrohemodynamics, hyperoxia attenuated the decrease in pulmonary capillary BFV and capillary shear rates. Attenuation of the decrease in microvascular shear rates may be an important mechanism of the beneficial effects of hyperoxia on reduction in leukocyte rolling adhesion and sequestration in the tissues. Although significant, it is not clear whether the magnitude of the changes induced by oxygen in BFV and shear rate is sufficient to explain the rather dramatic effect of this treatment on the local splanchnic and remote pulmonary responses.

When considering possible mechanisms other than microhemodynamic changes, it has been shown that a rapid decrease in endothelial nitric oxide synthase activity after I/R promotes leukocyte adherence, activation, and migration across the endothelial barrier and increases microvascular permeability, thus enhancing the tissue inflammatory reaction (13, 23). In contrast, increased endogenous nitric oxide generation may attenuate leukocyte and platelet aggregation and prevent the characteristic increased vascular permeability during I/R. Therefore, in light of the observations made by Miralles et al. (26) that high O2 tensions significantly induced inducible nitric oxide synthase mRNA and protein in the rat liver, one may suggest that this may be one of the possible anti-inflammatory mechanisms responsible for decreased leukocyte adhesion observed in the mesenteric microcirculation and the attenuated inflammatory response in the lungs. However, the time frame of the present experiments, in which marked microvascular inflammatory responses occurred very rapidly and in which significant effects of hyperoxia started after <1 h, is inconsistent with mechanisms that require at least a few hours (e.g., induction of mRNA and new protein synthesis) to be operative.

In this study normobaric hyperoxia also exerted a striking beneficial effect on plugging of mesenteric microvessels, maintaining it at very low values not significantly different from those in sham-operated animals. It is tempting to suggest that decreased leukocyte rolling, adhesion, and activation is the sole mechanism of this prominent observation. However, the exclusive role of leukocyte-endothelial interaction and leukocyte plugging in the “no-reflow” phenomenon is debated. It seems that at least in some tissues (e.g., skeletal muscle) other mechanisms such as disruption of endothelial integrity, with shift of fluids into endothelial cells, are associated with intravascular hemoconcentration, endothelial cell swelling, and interstitial edema formation, which contribute to capillary luminal narrowing and impairment of perfusion (25). In any event, it is suggested that the almost total prevention of plugging of ~60% of the microvessels is a key mechanism of the salutary effect of oxygen in splanchnic I/R.

Remote involvement of the lungs in the SIR after splanchnic I/R is an important feature of this condition and a crucial determinant of its outcome. The mechanisms by which the lungs are affected after splanchnic damage are primarily attributed to deleterious influences of activated leukocytes on the pulmonary microcirculation (31, 33, 36). There are two principal mechanisms by which leukocytes may be trapped in the microcirculation of the lungs, either as preactivated cells that are trapped in the capillary network of remote organs or as nonactivated cells that adhere to activated endothelial cells in the microcirculation. It is certainly possible that a combination of the two processes occurs as well. Entrapment of circulating leukocytes, not only in the target organ but also in remote organs, is widely accepted as a most important mechanism of multiorgan failure in a variety of conditions that initiate SIR including circulatory shock, sepsis, multiple trauma, and splanchnic ischemia.

In our experiments I/R of the SMA caused significant reduction in capillary BFV, decrease in circulating leukocytes, sequestration of WBC in the lungs, and marked increase in macromolecular flux into the pulmonary interstitium and alveolar spaces. Inhalation of oxygen attenuated the drop in capillary BFV, blunted a decrease in circulating WBC, decreased sequestration of leukocytes in the lungs, and prevented the increase in macromolecular flux to extravascular spaces and alveoli. This prominent effect of hyperoxia on the remote pulmonary inflammatory response after splanchnic ischemia may be related to attenuation of proinflammatory signals from the damaged intestines due to improved general hemodynamic status or to local hemodynamic or anti-inflammatory actions of hyperoxia in the lungs. In this regard, it was shown previously that hyperbaric oxygen reduces neutrophil preactivation and pulmonary leukosequestration after intestinal I/R (34). Hyperoxia causes pulmonary vasodilation and decreases pulmonary arterial pressure, especially in...
conditions characterized by hypoxemia and pulmonary hypertension. We did not measure pulmonary arterial pressure in the present experiments. It is certainly possible that decreased pulmonary vascular pressure could be one of the mechanisms by which hyperoxia attenuated microvascular leakage in our study. To the best of our knowledge, our results are the first demonstration of local and remote anti-inflammatory effects of normobaric hyperoxia after splANCHIC I/R. Because normobaric hyperoxia is easily attainable in the usual clinical setting, it is suggested that inhalation of 100% oxygen may be a useful adjunct to the therapy of splanchIC I/R.

DISCLOSURES

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