Microcirculatory effects of local and remote ischemic preconditioning in supraceliac aortic clamping

Nilon Erling Jr, MD,[a] Naomi Kondo Nakagawa, PhD,[b] José Walber Miranda Costa Cruz, PhD,[b] Fernando Luiz Zanoni, MVD,[b] José Carlos Costa Baptista-Silva, MD,[a] Paulina Sannomiya, PhD,[b] and **Luiz Francisco Poli-de-Figueiredo, MD,[c]** *São Paulo, SP, Brazil*

Introduction: **Supraceliac aortic clamping in major vascular procedures promotes splanchnic ischemia and reperfusion (I/R) injury that may induce endothelial dysfunction, widespread inflammation, multiorgan dysfunction, and death. We tested the hypothesis that local or remote ischemic preconditioning (IPC) may be protective against injury after supraceliac aortic clamping through the modulation of mesenteric leukocyte-endothelial interactions, as evaluated with intravital microscopy and expression of adhesion molecules.**

Methods: **Fifty-six male Wistar rats (weight, 190 to 250 g), were divided into four groups of 14 rats each: control—sham surgery without aortic occlusion; I/R through supraceliac aortic occlusion for 20 minutes, followed by 120 minutes of reperfusion; local IPC through supraceliac aortic occlusion for two cycles of 5 minutes of ischemia and 5 minutes of reperfusion, followed by the same protocol of the IR group; remote IPC through infrarenal aortic occlusion for two cycles of 10 minutes of ischemia and 10 minutes of reperfusion, followed by the same protocol of the IR group. Seven animals per group were used to evaluate in vivo leukocyte-endothelial interactions in postcapillary venules with intravital microscopy and another seven animals per group were used to collect mesentery samples for immunohistochemistry demonstration of adhesion molecules expression.**

Results: **Supraceliac aortic occlusion increased the number of rolling leukocytes with slower velocities and increased the number of adherent leukocytes to the venular surface and leukocyte migration to the interstitium. The expression of P-selectin, E-selectin, and intercellular adhesion molecule-1 was also increased significantly after I/R. Local or remote IPC reduced the leukocyte recruitment in vivo and normalized the expression of adhesion molecules.**

Conclusions: **Local or remote IPC reduces endothelial dysfunction on mesenteric microcirculation caused by I/R injury after supraceliac aortic clamping. (J Vasc Surg 2010;52:1321-9.)**

Clinical Relevance: **The present study demonstrates that ischemia and reperfusion injury induced by supraceliac aortic occlusion promotes endothelial dysfunction and leukocyte recruitment on mesenteric microcirculation. Local and remote preconditioning reduced leukocyte-endothelial interactions and normalized the expression of endothelial adhesion molecules involved in this process. Although we recognize the limitation of an experimental model, our findings suggest that local and remote ischemic preconditioning minimize the endothelial dysfunction and leukocyte recruitment events that play a central role in systemic inflammation and multiorgan dysfunction after major aortic reconstructions.**

Acute tissue ischemia has been recognized as a major cause of morbidity and mortality in patients who require supraceliac aortic clamping. Reperfusion, despite being essential to retain organ integrity, is also distinguished as a damaging process that magnifies the initial ischemic insult and results in widespread inflammation and multiorgan dysfunction.

From the Department of Surgery, Federal University of São Paulo,[a] Institute of Heart (InCor), LIM 11, University of São Paulo Medical School,[b] Department of Surgery, LIM 26, University of São Paulo Medical School.[c]

Supported by a grant from the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) 04/15964-6.

Competition of interest: none.

Reprint requests: Nilon Erling Jr, Rua Casemiro de Abreu, 908/202, 90420-000 Porto Alegre-RS, Brazil (e-mail: nilonjr@gmail.com).

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a competition of interest.

0741-5214/$36.00

doi:10.1016/j.jvs.2010.05.120

The pathophysiology of ischemia and reperfusion (I/R) injury is complex. Different pathways converge to microcirculatory derangements and acute inflammatory reactions[1] through the recruitment of circulating leukocytes in a multistep process within the postcapillary venules.[2] The activated endothelial cells promote leukocyte rolling and subsequent adhesion mediated largely by means of P- and E-selectin and intercellular adhesion molecule-1 (ICAM-1), respectively.[3] The rolling and adhesion are fundamental steps for the leukocyte transmigration and the amplification of inflammatory response.[4,5] The I/R injury affects leukocyte behavior, observed in intravital microscopy preparations and increases the expression of endothelial adhesion molecules.[3,6]

One of the most studied forms to mitigate the I/R injury is ischemic preconditioning (IPC). It consists of brief periods of ischemia and reperfusion before the more prolonged transient ischemia. In its classic form, the preconditioning stimulus is applied directly to the same tissue that will sustain the I/R lesion.[7] Another way in which IPC can

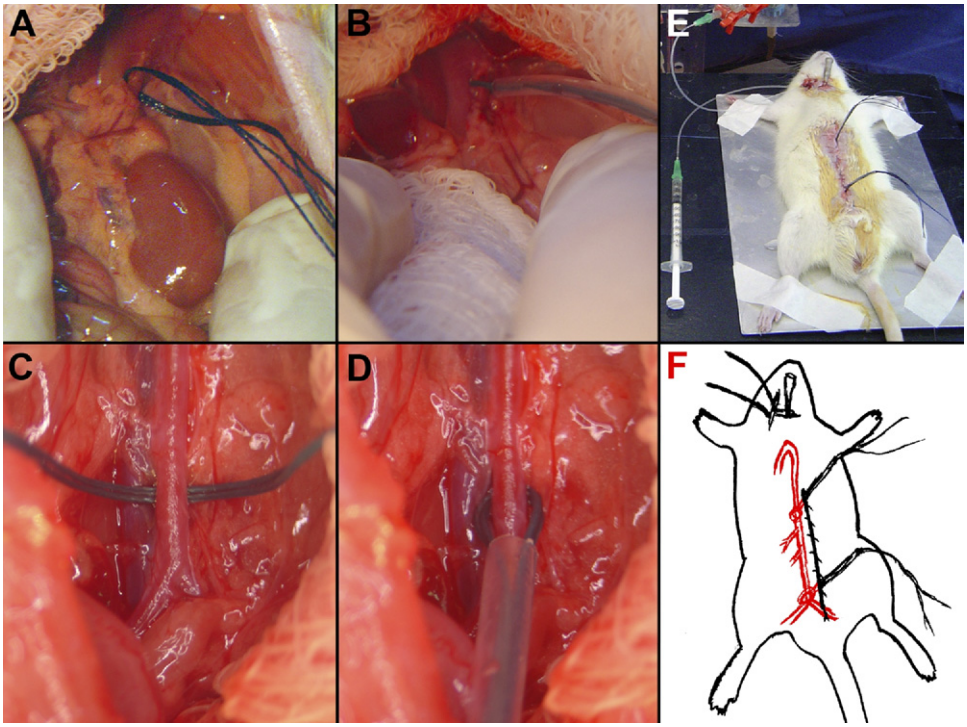

Fig 1. Dissection of abdominal aorta: **(A)** Supraceliac aortic control, for **(B)** tourniquet formation, and **(C)** infrarenal aortic control, for **(D)** tourniquet formation. **E,** During the experiment, the animal was placed on a heated platform. **F,** Illustration shows the final aspect of surgical preparation with the abdominal wall closed and Rumel tourniquets brought out of the wound to allow aortic occlusion and release without mesentery exposure.

be achieved is for the preconditioning stimulus to be applied in a distant tissue within the same[8] or in a different organ.[9] These IPC maneuvers, either local or remote, are particularly applicable to surgical patients who will undergo invasive or open surgical interventions.[10]

Compromise of mesentery perfusion has been implicated as a central event in multiorgan dysfunction after sepsis and in different shock status.[11,12] The microcirculatory effects of multivisceral I/R due to aortic cross-clamping are not clear, and the effects of IPC to mesentery microcirculation in this setting are not defined. The aim of this study was to test the hypothesis that local or remote IPC may provide protection against I/R injury in rats undergoing supraceliac aortic occlusion through the modulation of mesenteric leukocyte-endothelial interactions evaluated with intravital microscopy and expression of adhesion molecules.

MATERIAL AND METHODS

The experimental protocol in this study was approved by the Ethical Committee of Federal University of São Paulo and was performed according to National Institutes of Health *Guide for the Care and Use of Laboratory Animals.*

Animal model and surgical preparation. Fifty-six male Wistar rats (weight, 190-250 grams) were fasted overnight before the procedure, with free access to water. Anesthesia was induced with intraperitoneal sodium pentobarbital (50 mg/kg). Through a right anterior cervical incision, a tracheostomy was performed to allow airway control, spontaneous breathing, and secretions removal. The common carotid artery and external jugular vein were dissected and cannulated with polyethylene catheters. Venous access was used to inject solutions. Arterial access was used to monitor mean arterial pressure (MAP; MP 100, Biopac System Inc, Goleta, Calif).

Through a midline abdominal incision, the aorta was dissected and controlled proximally at the supraceliac portion between the diaphragmatic crura (Fig 1, *A*) and distally at the aortic bifurcation (Fig 1, *C*). The strings of the aortic control were used to create a 4-cm-long Rumel tourniquet (Fig 1, *B* and *D*). The abdominal wall was sutured, and these tourniquets were exteriorized at the top and at the bottom of the wound to allow aorta occlusion and release during the experiment (Fig 1, *E* and *F*). The abdominal wall was closed to reduce ambient exposure and manipulation of the mesentery to minimize basal inflammation, which could bias our results. The aortic occlusion and release necessary in some experimental groups was confirmed by an abrupt rise and fall of the MAP. Heparin was administered intravenously (100 IU/kg), and the animals were kept warm during the experiment with a heated platform set to 98.6°F on top of which a metallic plate was placed with the animal laying on it.

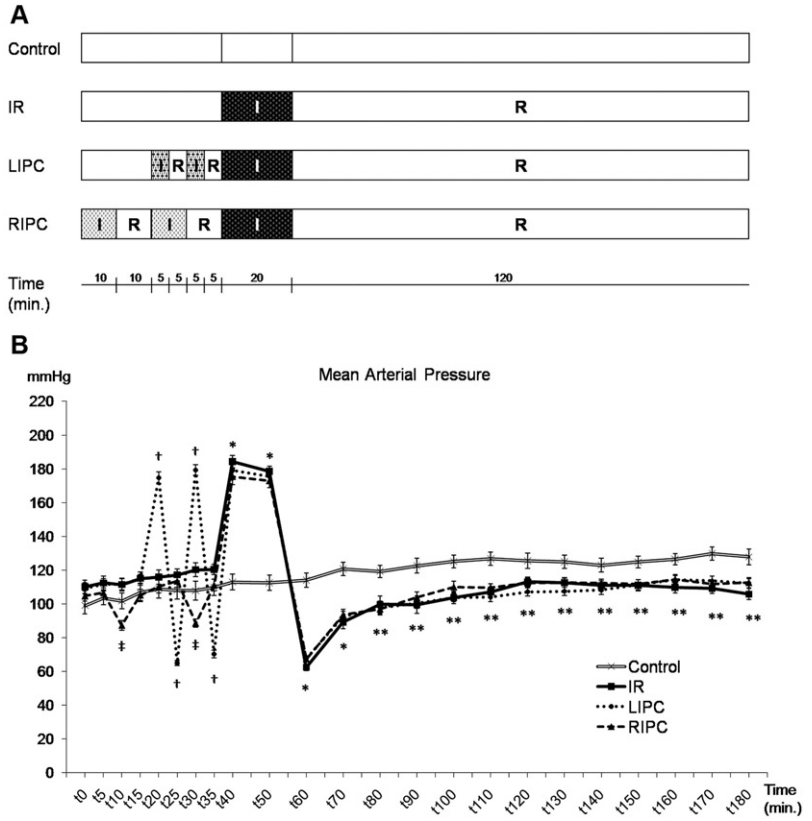

Fig 2. A, Experimental protocol and **(B)** main arterial pressure variations during the experiment in control, ischemia and reperfusion (*IR*), local ischemic preconditioning (*LIPC*), and remote ischemic preconditioning (*RIPC*) groups. Data expressed as mean ± standard error for 14 animals per group. *$P < .001$: IR, LIPC, and RIPC vs control. †$P < .001$: LIPC vs control. ‡$P < .05$: RIPC vs control.

Experimental design and study groups. After the same initial surgical preparation and stabilization, the animals were allocated into four experimental groups according to the duration of I/R. The control group was kept at rest during the entire experiment, without aortic occlusion. The IR group underwent 20 minutes of supraceliac aortic occlusion, followed by 120 minutes of reperfusion. The local IPC group (LIPC) underwent two cycles of supraceliac occlusion (5 minutes of ischemia and 5 minutes of reperfusion), followed by 20 minutes of supraceliac aortic occlusion and 120 minutes of reperfusion. The remote IPC (RIPC) group underwent two cycles of infrarenal occlusion (10 minutes of ischemia and 10 minutes of reperfusion), followed by 20 minutes of supraceliac aortic occlusion and 120 minutes of reperfusion (Fig 2, *A*). Seven animals from each group were used for intravital microscopy study and another seven for immunohistochemistry analysis.

The hemodynamic stability at the supraceliac clamp opening (occluded for 20 minutes) was accomplished by an infusion of 1.5 mL of saline 0.9%. Subsequent doses of 0.5 mL of saline 0.9% were administered after 30, 60, 90, and 120 minutes of reperfusion. This volume expansion was provided at the same time for all four groups. At the end of the experiment, the animals were exsanguinated by aortic puncture.

Direct vital microscopy of mesenteric microcirculation. Microcirculatory blood flow as well as leukocyte-endothelial cell interaction was evaluated through intravital microscopy at the end of the experiment (≤2 hours of reperfusion) by an independent investigator blinded to the study group. The previously sutured abdominal incision was opened and the mesentery was exposed for the in vivo examination. The animals were maintained on a special board warmed by circulating water at 37°C that also included a transparent platform on which the mesentery was positioned. During the period of intravital observation, the exposed mesentery surface was kept moist by continuously dripping warmed (37°C) Krebs-Henseleit solution (113 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L $CaCl_2$. $2H_2O$, 25 mmol/L $NaHCO_3$, 1.1 mmol/L $MgSO_4$, 1.1 mmol/L KH_2PO_4, and 5 mmol/L glucose, pH 7.20-7.40) saturated with a mixture of gases (95% N_2 and 5% CO_2) in an attempt to sustain microcirculatory characteristics unchanged throughout the intravital microscopic analysis.

The mesenteric microcirculation was assessed after 10 minutes of stabilization. Three to five postcapillary venules

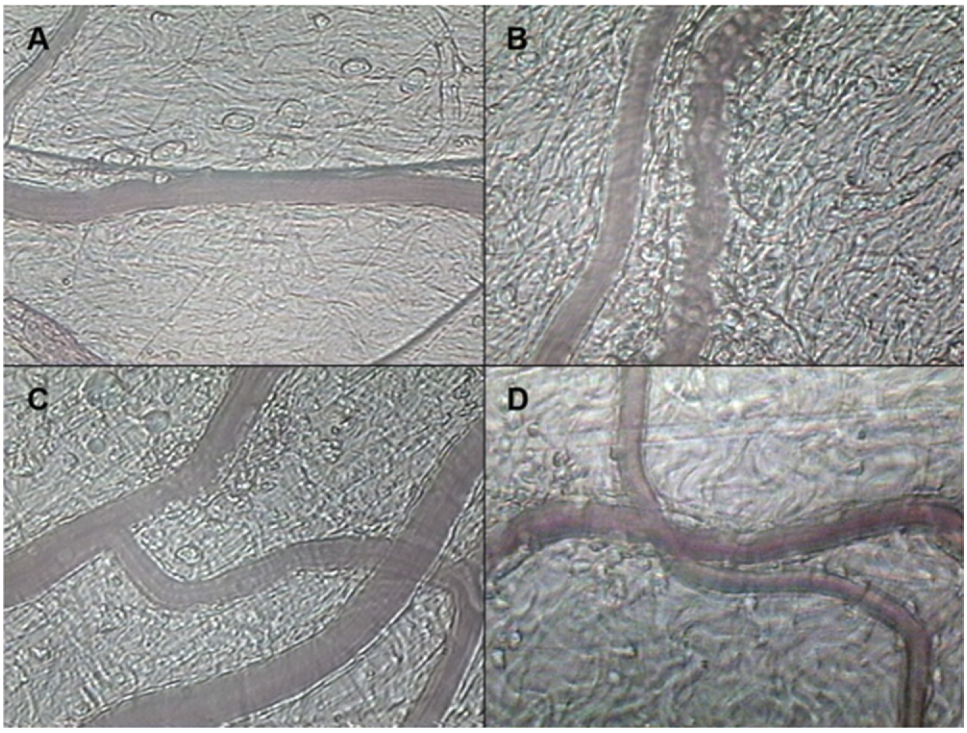

Fig 3. Representative intravital photomicrographs of mesenteric postcapillary venules (original magnification ×100) from **(A)** control, **(B)** ischemia and reperfusion (I/R), **(C)** local ischemic preconditioning (LIPC), and **(D)** remote ischemic preconditioning (RIPC) groups.

(diameter 15-25 μm) with corresponding adjacent interstitium were selected for each animal (Fig 3). A charge-coupled device color camera (TK-C1380U, JVC Co, Tokyo, Japan) was incorporated to a trinocular microscope (Axioplan 2; Carl Zeiss Co, München-Hallbergmoos, Germany) to facilitate the observation of the enlarged image (original magnification ×425) on a microcomputer monitor (SyncMaster 753DFX; Samsung, Manaus, MA, Brazil). Analyses of leukocyte-endothelium interactions were performed online by using Axiovision 4.1 image-computer software (Carl Zeiss Co) with an incorporated modulus of interactive measurements and time laps. Images were stored, enabling off-line playback analysis.

Leukocyte behavior

Rolling leukocytes. Rolling leukocytes were defined as white blood cells that moved significantly slower than the erythrocytes in a given microvessel. The number of rolling leukocytes was presented as the mean number of cells passing at a designated line perpendicular to the venular axis per 10 minutes. A given section of the vascular bed was tested only once. Three to five microvessels were randomly selected on a single animal to avoid improper sampling variability due to any flow disturbance. The individual value of a given animal for rolling leukocytes was the mean of these vessels' observations (3, 4, or 5), and the final mean and standard error of this variable was derived from just one value for each animal. Individual leukocyte rolling velocity was calculated from the time required for a steady rolling leukocyte to travel a defined distance in the microvessel.

Rolling velocity in each vessel was determined as the average velocity of 10 leukocytes. The same three to five microvessels were tested on a single animal to avoid improper sampling variability due to any flow disturbance. Individual value of a given animal for leukocyte rolling velocity was the mean of these vessels' observations (3, 4 or 5), and the final mean and standard error of this variable was derived from just one value for each animal. Results are presented as micrometers per second.

Adherent leukocytes. A leukocyte was considered to be adherent to the venular endothelium if it remained stationary for more than 30 seconds. Adherent cells were counted during a 10-minute period in a 100-μm segment of the vessel. Three to five microvessels were randomly selected on a single animal to avoid improper sampling variability due to any flow disturbance. The individual value of a given animal for adherent leukocytes was the mean of these vessels' observations (3, 4 or 5), and the final mean and standard error of this variable was derived from just one value for each animal.

Migrated leukocytes. The number of leukocytes accumulating at the connective tissue, randomly selected adjacent to the chosen postcapillary venule, was determined in a standard area of 5000 μm^2. Three to five different fields were evaluated for each microvessel to avoid improper sampling variability due to any previous flow disturbance.

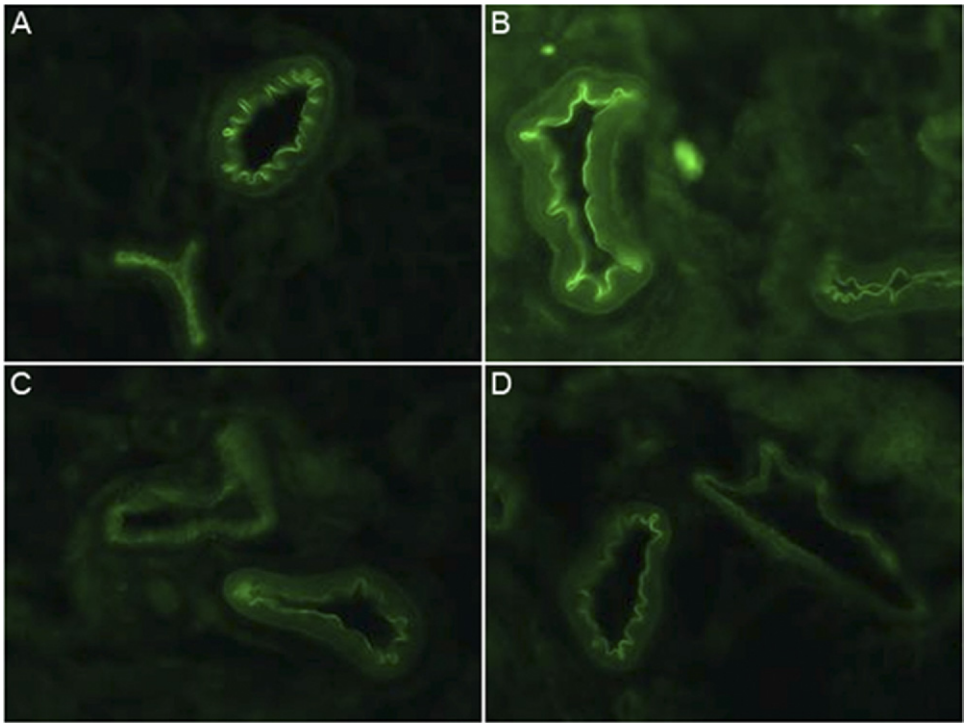

Fig 4. Representative photomicrographs of E-selectin immunofluorescence (original magnification ×1000) from **(A)** control, **(B)** ischemia and reperfusion (I/R), **(C)** local ischemic preconditioning (LIPC), and **(D)** remote ischemic preconditioning (RIPC) groups.

The individual value of a given animal for migrated leukocytes was the mean of these field observations (3, 4, or 5), and the final mean and standard error of this variable was derived from just one value for each animal. Three to five microvessels were selected on a single animal to avoid sampling variability.

Immunohistochemistry. At the end of the experiment, animals were exsanguinated by aortic puncture. The mesentery was dissected from the intestines and immersed in hexane while freezing into liquid nitrogen. Immunohistochemistry was chosen to evaluate the expression of adhesions molecules and localize it in the mesenteric microvessel. An independent investigator blinded to the study group performed this analysis.

Serial 8-μm cryostat sections were adhered to glass slides previously coated with organosilane (Sigma Chemical Co, St. Louis, Mo). For the immunodetection of ICAM-1, P-selectin, and E-selectin (Santa Cruz Biotechnology Inc, Santa Cruz, Calif), samples were fixed in acetone and exposed to 3% hydrogen peroxide. SuperBlock buffer (Pierce Biotechnology, Rockford, Ill) was used to block nonspecific sites. Tissue sections were incubated overnight at 4°C with a biotin-conjugated antirat immunoglobulin G antibodies against ICAM-1, P-selectin, and E-selectin. All these antibodies were diluted 1:50 in phosphate buffered saline (PBS) containing 0.3% Tween 20. After being rinsed in PBS three times for 10 minutes, sections were incubated with streptavidin-fluorescein (Amersham Pharmacia Biotech, London, UK; diluted 1:200) for 1 hour at room temperature. Samples were washed three times with PBS were treated with Vectashield mounting medium containing propidium iodide (Vector Labs, Burlingame, Calif) to preserve the fluorescence (Fig 4). Negative control samples were incubated with PBS instead of the primary antibody. Analyses were performed by using Image-Pro Plus 4.1 software (Media Cybernetics, Silver Spring, Md), which measures the intensity levels of fluorescence within the image. Results are presented as mean fluorescence intensity in units.

Statistical analysis. Data are expressed as mean ± standard error of the mean. Paired-sample *t* test was used for repeated measurement variables. Multiple comparisons between groups were performed using one-way analysis of variance, with post hoc analysis with Tukey, Tamhane, or Dunnett T3 tests, according to the Levene test of homogeneity of variance. Significance was set at $P < .05$.

RESULTS

Hemodynamic. At baseline, MAP was similar between groups (105.7 ± 1.9 mm Hg, $P = .177$). Supraceliac aortic occlusion always resulted in a significant MAP increase compared with baseline values, the preceding time, or the control group corresponding time ($P < .001$). Release of supraceliac aortic occlusion always caused a significant MAP decrease to values below the baseline or control group corresponding time ($P < .001$). Infrarenal

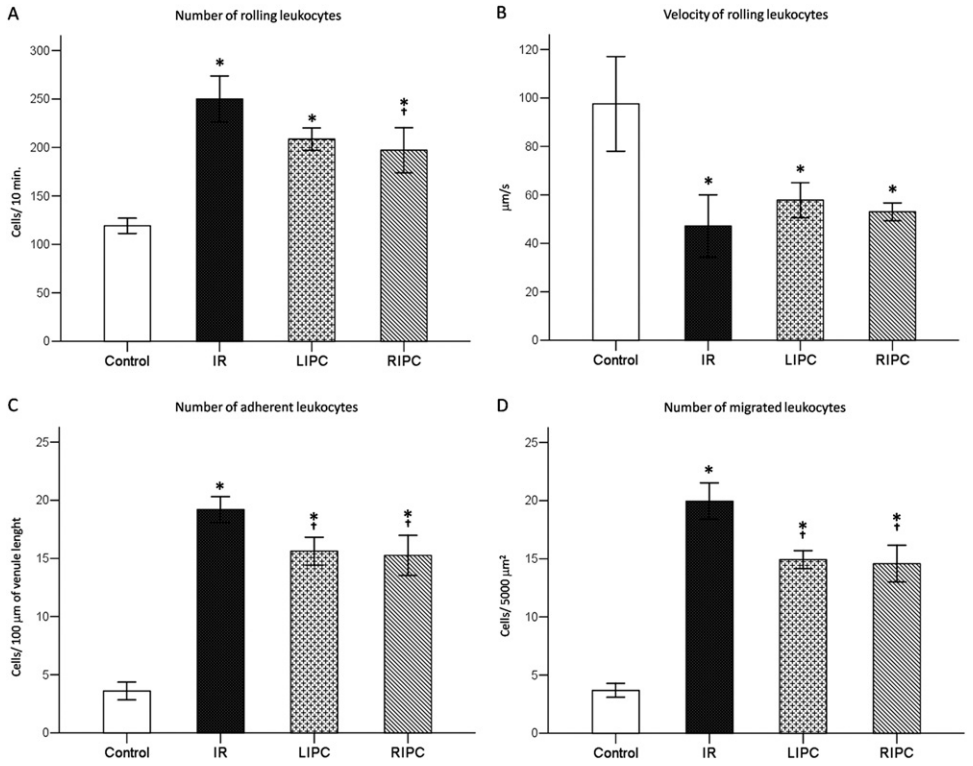

Fig 5. Mesenteric leukocyte-endothelium interaction. **A,** Number of rolling leukocytes, **(B)** velocity of rolling leukocytes, **(C)** number of adherent leukocytes, and **(D)** number of migrated leukocytes in control, ischemia and reperfusion *(IR)*, local ischemic preconditioning *(LIPC)*, and remote ischemic preconditioning *(RIPC)* groups. Data expressed as mean ± standard error for seven animals per group. *$P < .05$ vs control. †$P < .05$ vs IR.

aortic occlusion in the group did not increase MAP, but release caused a transient significant decrease in MAP ($P < .05$; Fig 2, *B*).

Mesenteric leukocyte-endothelium interactions. The mean diameter of unbranched postcapillary venules was 18.44 ± 0.37 μm and did not differ among groups ($P = .275$). The number of leukocytes rolling along the endothelial surface was 119.14 ± 3.91 cells/10 minutes in control group. The IR group increased more than twofold in this number (250.11 ± 11.85 cells/10 minutes, $P < .001$ vs control). The number of rolling leucocytes was raised in the LIPC, to 208.63 ± 5.84 cells/10 minutes ($P < .001$ vs control), and also in the RIPC group to 197.21 ± 11.62 cells/10 minutes ($P = .002$ vs control). Compared with the IR group, there was a significant reduction in the number of rolling leukocytes in the RIPC group ($P = .046$) but not in the LIPC group ($P = .072$; Fig 5, *A*). The leukocyte rolling velocity was 97.59 ± 9.77 μm/s in the control group. In the other three groups, there were significant reductions of velocities compared with the control group (IR: 47.15 ± 6.45 μm/s, $P = .008$; LIPC: 57.83 ± 3.60 μm/s, $P = .033$; and RIPC: 53.03 ± 1.8 μm/s, $P = .021$; Fig 5, *B*).

The number of adherent leukocytes was 3.60 ± 0.38 cells/100 μm venule length in the control group. The IR group had more than a fivefold increase in this number (19.20 ± 0.55 cells/100 μm venule length, $P < .001$). The LIPC and RIPC groups also raised the number of adherent leukocytes (LIPC: 15.63 ± 0.60 cells/100 μm venule length; RIPC: 15.26 ± 0.86 cells/100 μm venule length, both $P < .001$ vs control), but these boosts were significantly lower than in the IR group ($P = .002$ vs LIPC, and $P = .001$ vs RIPC; Fig 5, *C*). Leukocyte transmigration followed the same trend of leukocyte adherence. The number of migrated leukocytes was 3.67 ± 0.30 in controls, 19.96 ± 0.79 in IR, 14.92 ± 0.39 in LIPC, and 14.58 ± 0.79 cells/5000 μm^2 in RIPC. This phenomenon was significantly less intense in the control group than in the other groups ($P < .001$), and the number of migrated leukocytes in the LIPC and RIPC groups was significantly lower than in the IR group ($P < .001$; Fig 5, *D*).

Immunohistochemistry: Expression of P-selectin, E-selectin, and ICAM-1. Mesenteric expression of P-selectin almost doubled in the IR group compared with the control group (5.44 ± 0.69 vs 2.84 ± 0.36, $P = .015$). The fluorescence intensity of P-selectin was decreased in the LIPC (1.44 ± 0.29) and RIPC (1.33 ± 0.10) groups. These values were significantly lower than the IR group ($P < .000$) and were also lower than the control group ($P = .012$ vs LIPC, $P = .003$ vs RIPC; Fig 6, *A*). E-selectin expression was more than 2.6-fold more intense in the IR group than in the controls (6.86 ± 1.01 vs 2.62 ± 0.39,

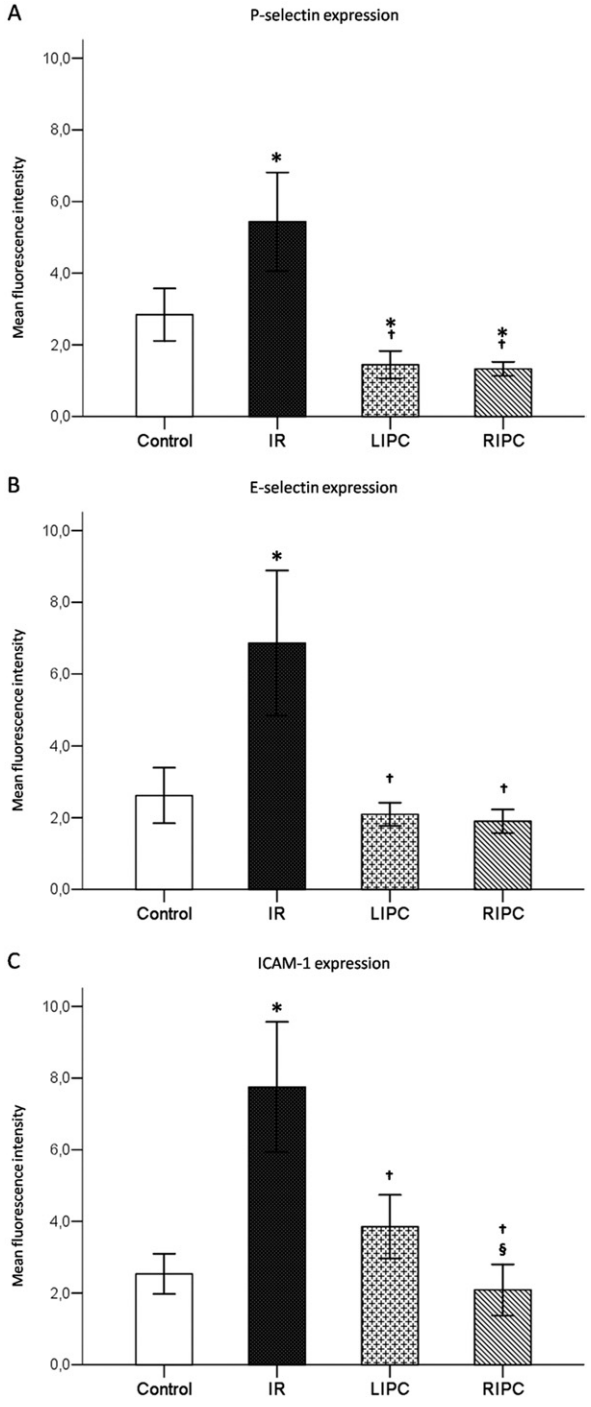

Fig 6. Expression of adhesion molecules **(A)** P-selectin, **(B)** E-selectin, and **(C)** intercellular adhesion molecule 1 *(ICAM-1)* in mesenteric endothelium in control, ischemia and reperfusion *(IR)*, local ischemic reperfusion *(LIPC)*, and remote ischemic preconditioning *(RIPC)* groups. Data are expressed as mean ± standard error for seven animals per group. *$P < .02$ vs control. †$P < .005$ vs IR. §$P < .03$ vs LIPC.

$P = .009$). In the LIPC and RIPC groups, this raise was abolished (2.09 ± 0.16 and 1.90 ± 0.16, respectively), rendering the fluorescence detection of E-selectin in these groups similar to the control group and significantly lower than the IR group ($P < .005$; Fig 6, *B*). ICAM-1 expression was amplified threefold in the IR group compared with the control group (7.75 ± 0.91 vs 2.53 ± 0.28, $P < .000$). The LIPC and RIPC groups did not show increased expression of ICAM-1, and it was lower in both of these groups than in the IR group ($P < .005$). In RIPC group the down-expression ICAM-1 was more pronounced than LIPC ($P = .022$; Fig 6, *C*).

DISCUSSION

This study characterized the microcirculatory response to I/R injury and the effect of local and remote IPC caused by supraceliac aortic occlusion in a clinically relevant experimental model. I/R injury is an integrant part of major vascular procedures. Despite great advances in less invasive endovascular techniques, complex aneurysmal disease involving the visceral aorta still requires surgical intervention.[13] Aortic occlusion at this level causes transient blood supply blockage to one or more visceral organs, and it is well recognized that this I/R injury is a very distressful situation.[14] Techniques used to decrease total ischemic time, such as distal aortic perfusion using left heart bypass, have proven to be effective in thoracoabdominal aortic aneurysm repair,[15,16] but morbidity and mortality of visceral aortic reconstruction remains high, especially if unselected data are considered.[17] The principal finding of this study is that local and remote IPC reduce the inflammatory response of I/R injury, documented in vivo by the down-regulation of the multistep process of leukocyte recruitment and also by the down-expression of endothelial adhesion molecules.

Microvascular dysfunction plays a central role in I/R injury and results from the amplified adhesive interactions between activated leukocytes and endothelial cells of postcapillary venules.[2] Different models of I/R injury demonstrated up-regulation of adhesion molecules P-selectin, E-selectin, and ICAM-1,[18,19] and the blockage or absence of one or more of these molecules causes a reduction of leukocyte accumulation.[20]

Our results demonstrated that isolated I/R injury increased the expression of P- and E-selectin. The IPC, either local or remote, conversely, led to a down-expression of these endothelial molecules. Interestingly, P-selectin expression in local or remote preconditioned animals was even lower than in controls. This finding, to some extent, contrasts with our intravital microscopic observations. The I/R injury increased the number of rolling leukocytes and decreased the rolling velocity, but the effect of local or remote IPC on that, despite being protective, was just marginally significant.

Supraceliac occlusion increased ICAM-1 expression, but not in preconditioned animals. The leukocyte behavior also follows this observation, with the more pronounced differences detected in the numbers of stickers and mi-

grated leukocytes, with a fivefold to sixfold increase in animals that underwent I/R injury. Local and remote preconditioning again effectively decrease these steps of leukocyte recruitment.

Some human observations are already available. IPC has long been established to exist in humans. Local IPC was able to preserve endothelial function and modulated inflammatory cell recruitment in volunteers who underwent forearm I/R,[21] what was later demonstrated to happen with contralateral arm (remote) IPC.[22] In clinical observations, local IPC reduced troponin T release 72 hours after coronary arterial bypass grafting (CABG).[23] During percutaneous coronary intervention (PCI), local IPC resulted in less ST-segment elevation, chest pain severity, regional wall motion abnormalities, and diastolic abnormalities.[24] Despite promising results, studies of local IPC did not fully achieve usefulness in the field of aortic surgery.

The less invasive nature of remote IPC makes it more amenable for tests in different clinical scenarios. Children undergoing repair of congenital heart defects were randomized to receive remote IPC using a lower limb blood pressure cuff or control treatment, and there was a decrease in postoperative levels of troponin I and inotrope requirements.[25] Remote IPC using upper limb blood pressure cuff also had a positive effect in adult patients undergoing CABG, reducing the overall serum troponin T serum concentration up to 48 hours after surgery.[26] In patients with abdominal aortic aneurysm, remote IPC was evaluated with temporary iliac clamping before the aortic repair.[10] This randomized controlled trial demonstrated a reduction in renal impairment and myocardial injury and also in infarction in preconditioned patients independent of other clinical or surgical covariables. A recently randomized trial of 242 consecutive patients showed that remote IPC, through the inflation of an upper arm pressure cuff, decreased the troponin I concentration at 24 hours in elective PCI and decreased the major adverse cardiac and cerebrovascular events at 6 months.[27]

The modifications induced by local and remote IPC are not totally clear. It is recognized that IPC confers different phases of protection. The early phase is independent of protein synthesis, with post-translational modifications. The late phase, known as second window of protection (SWOP), is mediated by synthesis of new proteins due to an altered gene expression.[28] Our model reproduces an acute situation like that of thoracoabdominal aortic repair, and it was not our intent to investigate the adaptive response of SWOP.

To our knowledge, this is the first investigation addressing microvascular dysfunction assessed with direct mesenteric intravital microscopy observation of leukocyte-endothelial interactions and evaluation of adhesion molecules expression in a proper model mimicking supraceliac aortic clamping to test the protective effect of local or remote preconditioning. In our experimental protocol, the I/R injury was achieved with 20-minute supraceliac occlusion, a period that allowed 120 minutes of reperfusion without death in our preliminary studies and was also applied by others.[29] Considering the lack of robust evidence and agreement about the more appropriate times of multivisceral local IPC or muscular remote IPC for supraceliac aortic occlusion, we used the most common and accepted protocols applied for intestine,[30] liver,[31] and skeletal muscle[10,32,33] to respect individual organ susceptibility to I/R injury. For local IPC, the great sensitiveness of intestine to I/R injury led to two 5-minute ischemia and 5-minute reperfusion cycles. For remote IPC, the better resistance of skeletal muscle to I/R injury led to two 10-minute ischemia and 10-minute reperfusion cycles.

Our experimental protocol has some limitations. We used healthy rats, without blood loss and fluid shifts that characterize aortic surgery in adults. Typically, cardiovascular, pulmonary, and renal dysfunctions are present in these patients and contribute substantially to the high rate of complications observed. Moreover, this is an acute protocol. Survival studies as well as larger animal models are needed to further evaluate organ dysfunction and long-term effects of the procedure. On the other hand, this well-standardized protocol allows us to demonstrate the effect of local and remote IPC on leukocyte-endothelial interactions with intravital microscopy that cannot be performed in humans. Our results support our hypothesis that IPC, either local or remote, may be beneficial to humans undergoing elective aortic repair.

CONCLUSIONS

Visceral and muscular ischemia caused by supraceliac aortic occlusion produces an intense inflammatory reaction in postischemic reperfused tissues. These changes modify leukocyte behavior and adhesion molecule expression. Local and remote IPC in this setting renders tissue more resistant to the deleterious effects of the prolonged I/R insult. Our study, in a proper model for aortic surgery, adds more evidence of the protective benefits of IPC. From a practical standpoint, these IPC maneuvers could be applied to humans, and this is particularly true for remote IPC, due to less risk of distal embolization from thrombus dislodgment or plaque fracture during arterial clamping, for example. Nevertheless, larger trials investigating IPC are still needed for a wider acceptance of this strategy to mitigate I/R injury and subsequent systemic inflammatory reaction as well as multiorgan dysfunction in major aortic surgery.

AUTHOR CONTRIBUTIONS

Conception and design: NE, PS, PF
Analysis and interpretation: NE, PS, PF
Data collection: NE, NN, JC, FZ
Writing the article: NE
Critical revision of the article: NE, JB, PS, PF
Final approval of the article: NE, PF
Statistical analysis: NE
Obtained funding: PF
Overall responsibility: NE

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Submitted Mar 15, 2010; accepted May 30, 2010.