Pretreatment Combination Reduces Remote Organ Damage Secondary to Intestinal Reperfusion Injury in Mice: Follow-up Study

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ABSTRACT

Background. Intestinal ischemia-reperfusion injury occurs after different surgical treatments, including intestinal transplantation. This harmful process may have an effect in remote organs, leading to multiple organ dysfunction syndrome and death. Therefore, to establish strategies to attenuate local and remote damage constitutes a challenge for experimental and clinical surgeons in the intestinal surgical field.

Methods. We evaluated the effect of ischemic preconditioning and tacrolimus pretreatment applied alone and in combination against local and remote damage caused by prolonged intestinal ischemia-reperfusion injury in a mouse model of warm ischemia.

Results. Ischemic preconditioning applied alone and in combination with tacrolimus decreased histological damage ($P < .05$), number of apoptotic cells ($P < .05$), nitrosative stress ($P < .01$), and serum lactate dehydrogenase activity ($P < .05$) and lowered uremia ($P < .05$) compared with untreated post-reperfused intestines. Regarding remote organ damage, combination therapy was the unique condition able to attenuate lung (mainly neutrophil infiltration and hemorrhage), liver (sinusoidal congestion and hepatic vacuolization), and kidney (acute tubular necrosis and hydropic degeneration) histological alterations ($P < .05$), compared with the untreated group.

Conclusions. These results support the application of these strategies in combination to minimize the impact of ischemia-reperfusion injury in the whole organism as a strategy to prevent multiple organ dysfunction syndromes and minimize the clinical impact.

ISCHEMIA-REPERFUSION INJURY (IRI) is produced on restoration of blood flow after an ischemic period. Different mechanisms contribute to local damage on re-oxygenation of ischemic tissue [1]. Furthermore, another consequence of IRI is the establishment of alterations in other organs (remote injury), a phenomenon that is observed for most tissues with ischemia-reperfusion events, including intestine, lung, liver, kidney, and heart [2]. The mechanisms involved in the development of remote lesions are partially similar to those producing local damage during IRI, mainly related to the release of different inflammatory mediators to the systemic compartment that target other remote effector organs. The reactive oxygen and nitrogen species, activated leukocytes, and inflammatory mediators such as cytokines and complement activation participate in the expansion of IRI to remote organs [3,4].

Particularly, intestinal IRI may alter the integrity of the mucosal barrier. Therefore, intraluminal bacteria and endotoxin lipopolysaccharide may pass through the mucosa into the systemic compartment, contributing directly and indirectly with the aforementioned remote organ damage [5,6]. The lungs are especially vulnerable to remote damage,

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particularly after events of hepatic or intestinal IRI, because in both cases the lungs represent the first major capillary bed through which blood passes from these organs.

In some situations, intestinal IRI is magnified and undertakes several organs and tissues, leading to a systemic inflammatory response syndrome (SIRS) that may lead to a multiple organ dysfunction syndrome (MODS), which may result in patient death [7]. Therefore, establishing strategies to mitigate IRI are major objectives for basic and translational research in the intestinal surgical field. Several strategies have been proposed to protect the intestine from IRI; however, there is no consensus on which is the most appropriate [8].

We previously reported that ischemic preconditioning (IPC), consisting of a short ischemic period followed by re-oxygenation and pretreatment using tacrolimus (TAC) alone, reduced intestinal IRI in the early stages of reperfusion, including intestinal histological damage, nitrosative stress, pro-inflammatory gene expression (interleukin [IL]-6 and IL-1β), and post-surgical survival [9]. However, the use of combined therapy (IPC+TAC) produced the most significant results in all evaluated parameters [9]. Despite these previous conclusions, the extent of damage from the onset of intestinal reperfusion until animal death and the alterations of remote organs were unclear. In this report, the impact of intestinal IRI to vital organs such as liver, lung, and kidney is reported.

METHODS

Animal Use and Care

Adult male Balb/c mice (average weight, 25 ± 3 g) were housed in a climate-controlled room on a 12-hour light-dark cycle, fed with standard laboratory mice chow, and allowed water ad libitum. Mice were provided by the School of Veterinary Sciences of the National University of La Plata, Argentina, animal facility. All of the experiments were performed according to the guidelines set by the National Institutes of Health (NIH publication volume 25, No. 28, revised 1996).

Surgical Procedure

A model of intestinal IRI in mice by reversible occlusion of the superior mesenteric artery (OSMA) was performed as previously described by our research group [10]. Mice were anesthetized with isoflurane (4% induction, 1.5% to 2% maintenance). Lidocaine (10 mg/kg) was placed into the skin and subcutaneous cellular tissue as a local anesthetic. Also, tramadol (20 mg/kg) was used for pain control.

Experimental Groups

Animals were divided into 5 groups of 5 animals each. In all groups except for the sham (SH) group, intestinal ischemia was performed for a period of 40 minutes.

In the control group (CT), OSMA was performed without treatment.

In the IPC group, a 10-minute cycle of intestinal ischemia followed by 10 minutes of reperfusion was performed before OSMA.

In the TAC group, intragastric TAC administration (3 mg/kg) was applied 12 hours before OSMA. The TAC dose was tested in previous studies published by our group. All animals not receiving TAC were administered the same volume of vehicle by gavage 12 hours before the procedure.

In the IPC+TAC group, both treatments were applied before OSMA in the same manner as performed in the IPC and TAC groups.

In the SH group, celiotomy and SMA dissection without OSMA was performed.

After the stipulated time of ischemia, intestines were reperfused. Four hours after onset of reperfusion, mice were killed by cervical dislocation, and sampling was performed. Distal jejunum, lungs, kidney, liver, and blood samples were taken for local and remote assessment of intestinal IRI.

Intestinal Histological Evaluation

Intestinal histological damage was evaluated by use of the Park Score as previously described [11]. Moreover, an immunohistochemical study to evaluate apoptosis was performed by use of the TUNEL technique, with the use of the In Situ Cell Death TMR (Roche), following the manufacturer's instructions. The counting of apoptotic cells was performed, considering the number of apoptotic cells in 10 fields per sample.

Nitrosative Stress Determination

To determine nitrosative stress, intestinal nitrite measurements were performed by use of the spectrophotometric method according to the technique described by Miranda et al [12] and modified by Beda and Nedospasov.

Remote Damage Assessment

Histological analysis of lung, kidney, and liver was performed by the pathology team on hematoxylin and eosin (H&E)-stained 5-μm tissue sections. All samples were analyzed by 2 experienced pathologists in a blinded fashion.

To evaluate lung damage, a previously described histopathology score was used [13]. Briefly, 5 parameters were considered for each condition analyzed: (1) neutrophil infiltration; (2) interstitial edema; (3) airway epithelial cell damage; (4) hyaline membrane formation; and (5) hemorrhage. Each parameter was scored (0, normal; 1, mild change; 2, moderate change; and 3, severe change). Each sample received a general score resulting from adding each evaluated parameter.

Hepatic histopathology evaluation was performed with the use of a previously described combined index [14]. Briefly, 3 parameters were considered for each condition: sinusoidal congestion, hepatocyte vacuolization, and the presence of edema. Each parameter was scored (0, no damage; 1, mild; 2, moderate and 3, severe changes), and each sample received a general score resulting from the addition of each evaluated parameter.

Renal damage was analyzed by means of a reported histopathology score [15]. Briefly, the following considerations were used to score each analyzed condition: normal kidney = 0 point; presence of acute tubular necrosis (ATN) less than 5% = 1 point; presence of ATN between 5% and 25% = 2 points; ATN greater than 25% = 3 points. The presence of hydropic degeneration added an extra point.

Plasma lactate dehydrogenase (LDH) levels were measured by use of a spectrophotometric technique with the use of a commercial kit. Plasma urea values were determined through the use of the ureasa color test (Wiener), following the manufacturer's instructions.

Statistical Analysis

Continuous variables were analyzed by means of 1-way analysis of variance, (ANOVA), followed by the Dunnett post-test. Discrete variables were analyzed by means of the Kruskal-Wallis test. Statistical analyses were performed with the use of GraphPad software version 5.00 (San Diego, Calif, United States).

RESULTS

Combined treatment was the most effective means to prevent intestinal damage.

Intestinal Nitrosative Stress

The CT group showed the highest levels of nitrites (2.5 ± 0.2 nmol/mg of tissue), followed by the TAC group (2.2 ± 0.1 nmol/mg). The IPC and IPC+TAC groups had an average of 1.7 ± 0.1 and 1.3 ± 0.1 nmol/mg, respectively. The SH group had the lowest values (0.7 ± 0.07 nmol/mg). Statistically significant differences ($P < .01$) were observed between the CT group versus the SH, IPC, and IPC+TAC groups (Fig 1A).

Intestinal Histological Damage and Apoptosis

Intestinal damage was scored according to the Park classification (Fig 1B). The CT group had an average of 3 ± 1.2, with a median of 3. The IPC and TAC groups showed an average of 1.2 ± 0.7 (median, 1) and 2 ± 0.4 (median, 0), respectively. All samples of the SH and IPC+TAC groups showed a normal intestine (Park classification, 0). Statistically significant differences ($P < .05$) were found between the CT group versus the SH, IPC, and IPC+TAC groups.

Apoptosis was evaluated by use of the TUNEL technique. All treated groups (IPC, TAC, and IPC+TAC) showed a significantly reduced number of apoptotic cells in comparison to the CT group ($P < .05$) (Fig 1C).

Remote damage was best prevented by combined IPC+TAC treatment.

Lung, Liver, and Kidney Histological Evaluation

Lungs of the CT group showed clear alterations, with an average damage score of 6.6 ± 1.8 (Fig 2A). The main lesions observed in this group were moderate to severe infiltration of neutrophils and moderate to severe hemorrhage (Fig 2B). The IPC and TAC groups had an average score of 1.6 ± 0.8 and 2.2 ± 1.3, respectively. In both groups, neutrophil infiltration (mild to moderate) was the most frequent alteration, and the effects of each treatment alone did not produce significant protection when compared with the CT group.

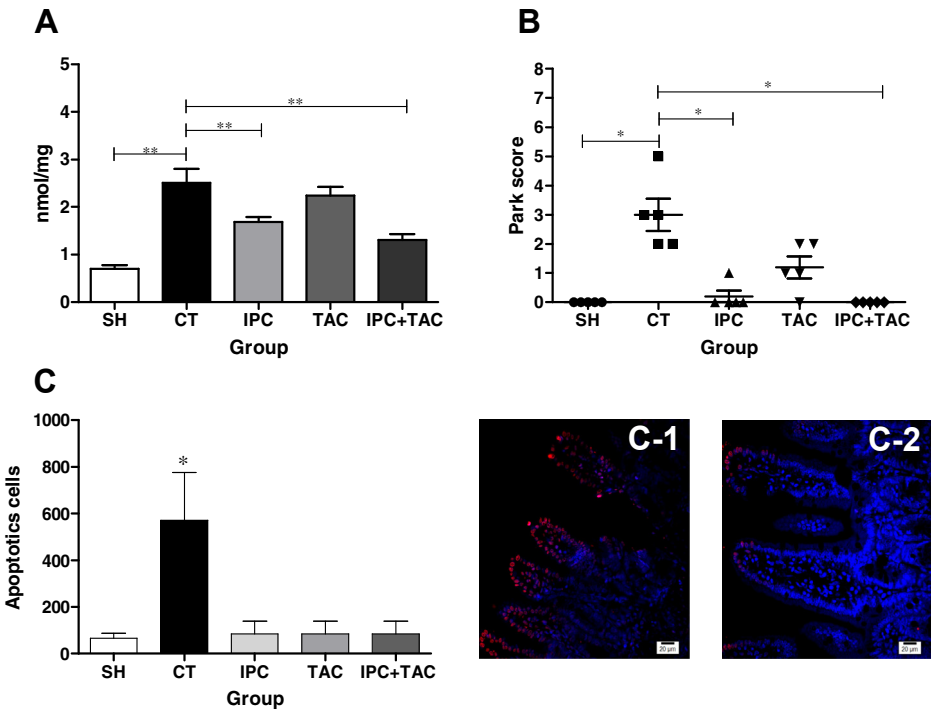

Fig 1. IPC alone and in combination with TAC attenuate nitrosative stress and intestinal histological damage after IRI. (**A**) Intestinal nitrite levels determined in organ lysate. $^{**}P < .01$, CT versus SH, IPC, and IPC+TAC groups (ANOVA). (**B**) Park Index at 4 hours of reperfusion in different groups (SH, sham group; CT, I/R control; IPC, ischemic preconditioned group; TAC, tacrolimus-treated group; IPC+TAC, ischemic preconditioned and tacrolimus-treated group). Each point represents an individual animal. $^{*}P < .05$, CT versus SH, IPC, and IPC+TAC groups (Kruskal-Wallis test). (**C**) Number of apoptotic cells determined by TUNEL assay and counted in 10 fields for the different groups (n = 5). $^{*}P < .05$, CT versus treated and SH groups (ANOVA). (**C-1**) Corresponds to the CT group; an increment of apoptotic cells located is at the tip of the villus in respect to treated groups, in which few apoptotic cells were observed (**C-2**).

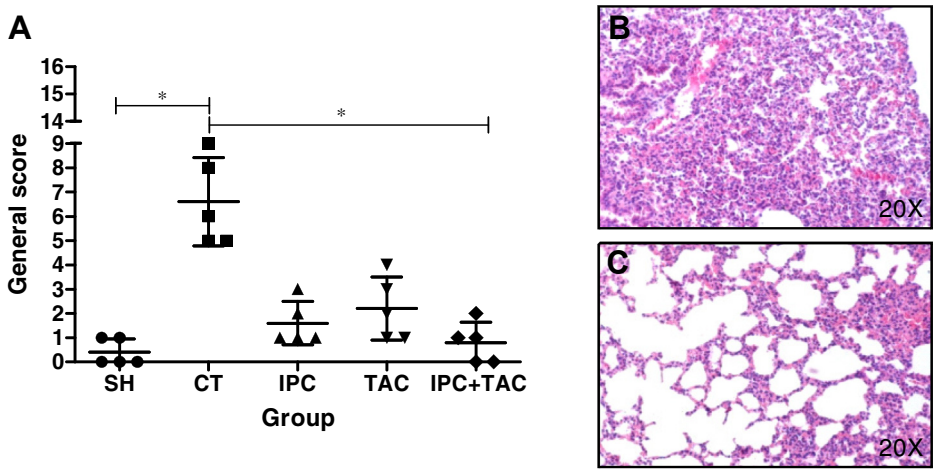

Fig 2. Combined therapy (IPC+TAC) attenuates histological lung damage caused by intestinal IRI. (**A**) Lung damage in each group (n = 5) was calculated by use of the standard histology score in a blinded manner. Groups are similar to those described in Fig 1. Significant differences were observed in the CT group versus IPC+TAC treatment. *$P < .05$ (Kruskal-Wallis test). Histological alterations in the CT group (**B**) and the IPC+TAC group (**C**) were observed by use of light microscopy (H-E staining).

Samples of the IPC+TAC group had histologically well-preserved lungs, with an average score of 0.8 ± 0.8 (Fig 2C), which was comparable with the SH group, which showed normal to mildly altered lungs. Consistently, significant differences ($P < .05$) were observed between the CT group versus the SH and IPC+TAC groups, indicating that the combination treatment was the only intervention that produced reversal of the effects on remote organs caused by the I/R treatment.

Microscopic evaluation of livers after intestinal IRI (Fig 3A) showed no abnormalities in the SH group. A mild to moderate sinusoidal congestion and hepatic vacuolization were the most remarkable lesions found in the CT group (Fig 3B). Besides, IPC and TAC treatments showed improved histology; however, still mild vacuolization of hepatocytes was observed as typical lesion. As in the case of lung damage, individual treatments did not produce statistically significant differences in the damage score when compared with the CT group. In the case of the IPC+TAC group, most liver samples showed no histological signs of damage (Fig 3C), and significant protection was observed (CT, $P < .05$ versus SH and IPC+TAC groups).

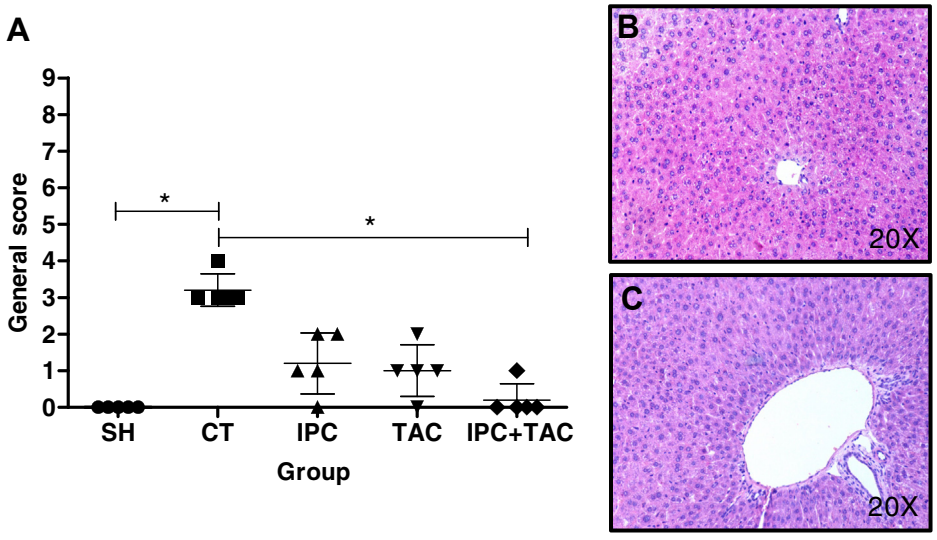

Fig 3. Pretreatment modulates liver injury after intestinal I/R event. Histological parameters were evaluated in a blinded manner (n = 5). Groups are similar to those described in Fig 1. (**A**) Statistically significant differences were observed between the CT group and the IPC+TAC group. *$P < .05$ (Kruskal-Wallis test). Images show the histological appearance of the liver in CT (**B**) and IPC+TAC (**C**) groups (H-E staining).

Regarding renal damage analysis (Fig 4A), all samples from the CT group showed hydropic degeneration and ATN less than 5% (3 samples) or between 5% and 25% (2 samples) (Fig 4B). The IPC and IPC+TAC groups had hydropic degeneration only (Fig 4C); therefore, the histological score was 1 in all cases. The TAC group showed an intermediate situation between the CT group and the IPC and IPC+TAC groups. Hydropic degeneration was observed in all samples, and ATN (<5%) was detected in 2 samples. Statistically significant differences ($P < .05$) were observed between the CT group versus the SH, IPC, and IPC+TAC groups.

Plasma LDH and Urea Levels

The LDH levels were measured as a marker of overall cellular damage. The CT group showed the highest values (CT, 1696.25 ± 339.4 mU/mL) and the SH group showed minimal LDH activity (SH, 550 ± 301 mU/mL). The other groups showed intermediate levels (IPC, 1188.7 ± 156.3; TAC, 1270 ± 450.9; and IPC+TAC, 912.5 ± 169.7 mU/mL) (Fig 5A). Only the IPC+TAC group had decreased LDH levels compared with the CT group ($P < .05$).

Urea plasma levels were measured as indicator of renal function. The values of each group were CT, 0.97 ± 0.1 g/L; IPC, 0.68 ± 0.2 g/L; TAC, 0.66 ± 0.2 g/L; and IPC+TAC, 0.42 ± 0.1 g/L (Fig 5B). Statistically significant differences ($P < .05$) were observed between the CT group versus the IPC+TAC groups.

DISCUSSION

It is known that the intestine is an important contributor for MODS, and intestinal IRI may trigger this syndrome, compromising the whole organism and survival [16]. Therefore, finding therapies to attenuate local and remote intestinal IRI is a subject of interest in the surgical field and in transplantation.

The use of TAC treatment to prevent intestinal damage on IRI has been previously shown to be successful by Oltean et al [17] in an intestinal transplantation rat model. Furthermore, the use of this immunosuppressor has an effect on preserving intestinal microcirculation that depends on its capacity to block NFkB signaling [18], although it may also depend on the dosing scheme and the IRI model used [19]. Furthermore, the use of intestinal ischemic preconditioning has also been shown to have protective effects on liver [20]. We have previously shown that the combination of IPC and TAC pretreatment has better performance in preventing intestinal damage in an intestinal I/R injury mouse model than any of the treatments alone. In the present study, we showed that the damage initiated in the intestine significantly affects remote organs such as lungs, liver, and kidney, and the combination of IPC and TAC pretreatment was the most efficient treatment to reduce remote injury, when compared with each treatment alone, reinforcing the concept of combined therapy.

The physiopathology of remote organ damage on intestinal IRI is complex and is fueled by 3 main mechanisms: (1) intestine-derived cytokines and activated cells that are released to internal milieu and spread by blood or the lymphatic system [21] and subsequently act directly on remote organs; (2) luminal microbiota or microbial-derived molecules that are translocated to circulation and activate an innate response in remote organs; and (3) as a tertiary mechanism, local organ-derived cytokines and local cell activation induced by the previously mentioned factors. Several sometimes overlapping pathways contribute to this mechanism, and the IPC+TAC preventive strategy used

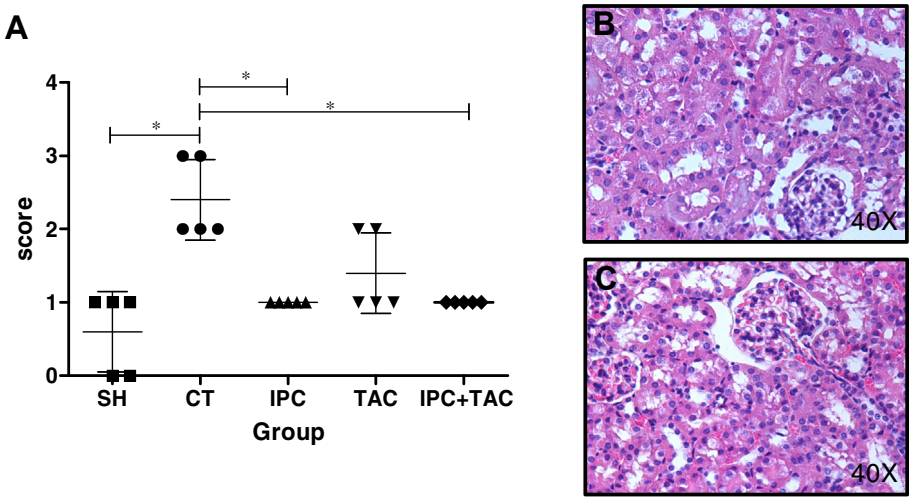

Fig 4. Renal damage induced by intestinal IRI was attenuated in IPC-containing groups. (**A**) Histological parameters were evaluated in a blinded manner (n = 5). Groups are similar to those described in Fig 1. *$P < .05$, CT versus SH, IPC, and IPC+TAC groups (Kruskal-Wallis test). (**B**) Corresponds to the CT group; hydropic degeneration and mild NTA are shown. (**C**) IPC and IPC+TAC groups (H-E staining).

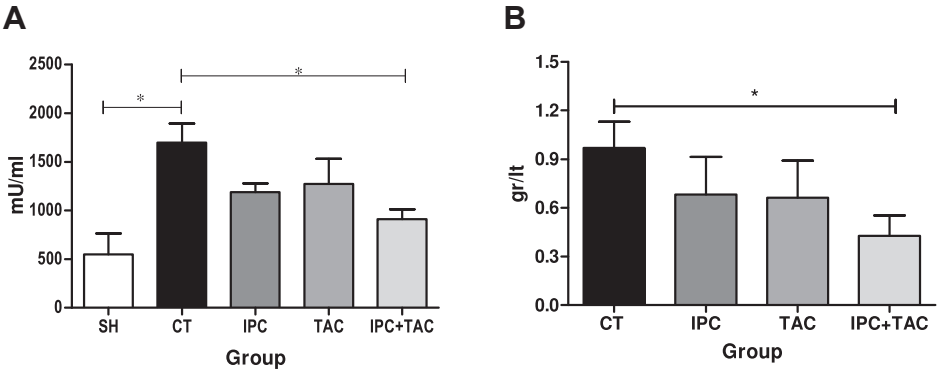

Fig 5. Combined pretreatment modulate LDH (**A**) and urea (**B**) plasma levels. Groups are similar to those described in Fig 1. In both cases, significant differences were observed between the CT group and the IPC+TAC group. *$P < .05$ (ANOVA).

here may modulate this phenomenon at several levels. The experiments shown here are not able to discriminate between these different possibilities or the individual role of any particular cytokine. The participation of tumor necrosis factor-α (TNF-α) as an inflammation-associated trigger in remote organ damage has been well documented [22,23]. Nevertheless, the use of gene-targeted KO animal models with loss of function of the TNF-α–TNF-SFR1 axis indicates that other redundant factors are implied [24]. Among cytokines, several others may mediate this effect. In our model, we have shown overexpression of IL-1b in intestine on IRI [9], whereas the related cytokine IL-18 has also been shown to participate in IRI damage in other organs [25], and its role in intestinal IRI remains to be established.

The participation of microbial-derived molecules on intestinal IRI has been clearly established [26], with multiple pathways implicated. Impairment of the intestinal barrier function at the molecular and histological levels correlates with bacterial products/bacterial translocation [27], and IPC has been shown to be effective in promoting this phenomena [28]. Maximal histological preservation shown on IPC+TAC treatment (Fig 1) contributes to minimization of microbial translocation. In particular, avoiding massive epithelial apoptosis as shown here (Fig 1) is important in lowering inflammatory effects [29]. The implication of TLR/MyD88 signaling in local and remote tissue damage on intestinal IRI has been recognized [30,31]. However, because these pathways are also involved in triggering several cellular activities, they not only enhance damage but they may contribute to homeostasis, depending on the context, because microbial-derived signals also participate in maintaining intestinal epithelial barrier [32,33]. Furthermore, damage-dependent inflammosome activation contributes to amplification of inflammatory signals independent of microbial products [34]. The IPC+TAC combined strategy presumably acts at different levels of these amplification cascades because it modulates inflammatory gene expression, with TAC treatment being the main driver of this effect [9]. Furthermore, IPC+TAC also minimizes intestinal barrier damage (Fig 1), with IPC treatment being mainly responsible of this latter effect. Consequently, the combination of both strategies is effective on the different actors mediating remote organ damage, resulting in lower remote organ alteration (Figs 2–4) and minimal overall cellular damage and loss of renal function (Fig 5), which was not observed for any individual intervention. Although IPC reduced the intestinal parameters evaluated and improved renal injury after I/R, the unique condition that showed best overall results in different remote organs evaluated was the application of both therapies (IPC+TAC) in combination (Figs 2–4).

The evidence presented here supports the application of these strategies to prevent intestinal IRI and its effects in the whole organism as a strategy to prevent MODS and minimize its clinical impact.

REFERENCES

[1] Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol 2012;298:229–317.

[2] Santora RJ, Lie ML, Grigoryev DN, et al. Therapeutic distant organ effects of regional hypothermia during mesenteric ischemia-reperfusion injury. J Vasc Surg 2010;52:1003–14.

[3] He GZ, Dong LG, Chen XF, et al. Lymph duct ligation during ischemia/reperfusion prevents pulmonary dysfunction in a rat model with omega-3 polyunsaturated fatty acid and glutamine. Nutrition 2011;27:604–14.

[4] de Arruda MJ, Poggetti RS, Fontes B, et al. Intestinal ischemia/reperfusion induces bronchial hyperreactivity and increases serum TNF-alpha in rats. Clinics (Sao Paulo) 2006;61:21–8.

[5] Lu YC, Yeh WC, Ohashi PS. LPS/TLR4 signal transduction pathway. Cytokine 2008;42:145–51.

[6] Wang Z, Hernandez F, Pederiva F, et al. Ischemic preconditioning of the graft for intestinal transplantation in rats. Pediatr Transplant 2011;15:65–9.

[7] Kinross JM, Darzi AW, Nicholson JK. Gut microbiome-host interactions in health and disease. Genome Med 2011;3:14.

[8] Mallick IH, Yang W, Winslet MC, Seifalian AM. Ischemia-reperfusion injury of the intestine and protective strategies against injury. Dig Dis Sci 2004;49:1359–77.

[9] Stringa P, Romanin D, Lausada N, et al. Ischemic preconditioning and tacrolimus pretreatment as strategies to attenuate intestinal ischemia-reperfusion injury in mice. Transplant Proc 2013;45:2480–5.

[10] Stringa P, Lausada N, Romanin D, et al. Defining the nonreturn time for intestinal ischemia reperfusion injury in mice. Transplant Proc 2012;44:1214–7.

[11] Park PO, Haglund U, Bulkley GB, Falt K. The sequence of development of intestinal tissue injury after strangulation ischemia and reperfusion. Surgery 1990;107:574–80.

[12] Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide Biol Chem 2001;5:62–71.

[13] Zhou H, Liu J, Pan P, et al. Carbon monoxide inhalation decreased lung injury via anti-inflammatory and anti-apoptotic effects in brain death rats. Exp Biol Med 2010;235:1236–43.

[14] Sano T, Izuishi K, Hossain MA, et al. Hepatic preconditioning using lipopolysaccharide: association with specific negative regulators of the Toll-like receptor 4 signaling pathway. Transplantation 2011;91:1082–9.

[15] Cicora F, Stringa P, Guerrieri D, et al. Amelioration of renal damage by administration of anti-thymocyte globulin to potential donors in a brain death rat model. Clin Exp Immunol 2012;169:330–7.

[16] Altshuler AE, Lamadrid I, Li D, et al. Transmural intestinal wall permeability in severe ischemia after enteral protease inhibition. PLoS One 2014;9:e96655.

[17] Oltean M, Pullerits R, Zhu C, et al. Donor pretreatment with FK506 reduces reperfusion injury and accelerates intestinal graft recovery in rats. Surgery 2007;141:667–77.

[18] Oltean M, Olofsson R, Zhu C, et al. FK506 donor pretreatment improves intestinal graft microcirculation and morphology by concurrent inhibition of early NF-kappaB activation and augmented HSP72 synthesis. Transplant Proc 2005;37:1931–3.

[19] Kalia N, Wood RF, Pockley AG, Brown NJ. Mucosal villus microcirculatory disturbances associated with rat intestinal ischaemia-reperfusion injury are not prevented by tacrolimus. Digestion 2003;67:154–60.

[20] Oltean M, Zhu C, Mera S, et al. Reduced liver injury and cytokine release after transplantation of preconditioned intestines. J Surg Res 2009;154:30–7.

[21] Cavriani G, Domingos HV, Soares AL, et al. Lymphatic system as a path underlying the spread of lung and gut injury after intestinal ischemia/reperfusion in rats. Shock 2005;23:330–6.

[22] Koksoy C, Kuzu MA, Kuzu I, et al. Role of tumour necrosis factor in lung injury caused by intestinal ischaemia-reperfusion. Br J Surg 2001;88:464–8.

[23] Sorkine P, Setton A, Halpern P, et al. Soluble tumor necrosis factor receptors reduce bowel ischemia-induced lung permeability and neutrophil sequestration. Crit Care Med 1995;23:1377–81.

[24] Soares AL, Coelho FR, Guabiraba R, et al. Tumor necrosis factor is not associated with intestinal ischemia/reperfusion-induced lung inflammation. Shock 2010;34:306–13.

[25] Wang J, Long Q, Zhang W, Chen N. Protective effects of exogenous interleukin 18-binding protein in a rat model of acute renal ischemia-reperfusion injury. Shock 2012;37:333–40.

[26] Vollmar B, Menger MD. Intestinal ischemia/reperfusion: microcirculatory pathology and functional consequences. Langenbecks Arch Surg 2011;396:13–29.

[27] Rosero O, Onody P, Kovacs T, et al. Impaired intestinal mucosal barrier upon ischemia-reperfusion: “patching holes in the shield with a simple surgical method”. Biomed Res Int 2014;2014:210901.

[28] Medeiros Ada C, Araujo-Filho I, Torres ML, et al. Ischemic preconditioning in different times and its effect on bacterial translocation induced by intestinal ischemia and reperfusion in rats. Rev Col Bras Cir 2013;40:55–9.

[29] Wu R, Dong W, Wang Z, et al. Enhancing apoptotic cell clearance mitigates bacterial translocation and promotes tissue repair after gut ischemia-reperfusion injury. Int J Mol Med 2012;30:593–8.

[30] Ben DF, Yu XY, Ji GY, et al. TLR4 mediates lung injury and inflammation in intestinal ischemia-reperfusion. J Surg Res 2012;174:326–33.

[31] Victoni T, Coelho FR, Soares AL, et al. Local and remote tissue injury upon intestinal ischemia and reperfusion depends on the TLR/MyD88 signaling pathway. Med Microbiol Immunol 2010;199:35–42.

[32] Chen LW, Chang WJ, Chen PH, et al. TLR ligand decreases mesenteric ischemia and reperfusion injury-induced gut damage through TNF-alpha signaling. Shock 2008;30:563–70.

[33] Watanabe T, Kobata A, Tanigawa T, et al. Activation of the MyD88 signaling pathway inhibits ischemia-reperfusion injury in the small intestine. Am J Physiol Gastrointest Liver Physiol 2012;303:G324–34.

[34] Muhlbauer M, Perez-Chanona E, Jobin C. Epithelial cell-specific MyD88 signaling mediates ischemia/reperfusion-induced intestinal injury independent of microbial status. Inflamm Bowel Dis 2013;19:2857–66.