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SCIENTIFIC ARTICLE

Pretreatment with remifentanil protects against the reduced-intestinal contractility related to the ischemia and reperfusion injury in rat

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KEYWORDS

Intestinal contractility;
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Abstract

Background and objectives: Serious functional and structural alterations of gastrointestinal tract are observed in failure of blood supply, leading to gastrointestinal dismotility. Activation of opioid receptors provides cardioprotective effect against ischemia–reperfusion (I/R) injury. The aim of the present study was to determine whether or not remifentanil could reduce I/R injury of small intestine.

Methods: Male Wistar Albino rats were subjected to mesenteric ischemia (30 min) followed by reperfusion (3 h). Four groups were designed: sham control; remifentanil alone; I/R control; and remifentanil + I/R. Animals in remifentanil + I/R group were subjected to infusion of remifentanil ($2 \mu\text{g kg}^{-1} \text{min}^{-1}$) for 60 min, half of which started before inducing ischemia. Collecting the ileum tissues, evaluation of damage was based on contractile responses to carbachol, levels of lipid peroxidation and neutrophil infiltration, and observation of histopathological features in intestinal tissue.

Results: Following reperfusion, a significant decrease in carbachol-induced contractile response, a remarkable increase in both lipid peroxidation and neutrophil infiltration, and a significant injury in mucosa were observed. An average contractile response of remifentanil + I/R group was significantly different from that of the I/R group. Lipid peroxidation and neutrophil infiltration were also significantly suppressed by the treatment. The tissue samples of the I/R group were grade 4 in histopathological evaluation. In remifentanil + I/R group, on the other hand, the mucosal damage was moderate, staging as grade 1.

Conclusions: The pretreatment with remifentanil can attenuate the intestinal I/R injury at a remarkable degree possibly by lowering lipid peroxidation and leukocyte infiltration.

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PALAVRAS-CHAVE

Contratibilidade intestinal;
Lesão intestinal de isquemia/reperfusão;
Rato;
Remifentanil

Pré-tratamento com remifentanil protege contra a redução da contratilidade intestinal relacionada à lesão de isquemia e reperfusão em ratos

Resumo

Justificativa e objetivos: Alterações funcionais e estruturais sérias do trato gastrointestinal são observadas na insuficiência de irrigação sanguínea, levando a alterações da motilidade gastrointestinal. A ativação dos receptores opioides proporciona um efeito cardioprotetor contra a lesão de isquemia/reperfusão (I/R). O objetivo do presente estudo foi determinar se remifentanil pode ou não reduzir a lesão de I/R do intestino delgado.

Métodos: Ratos machos albinos, da linhagem Wistar, foram submetidos à isquemia mesentérica (30 minutos) seguida de reperfusão (3 horas). Quatro grupos foram designados: *sham* controle; remifentanil isolado; controle I/R; remifentanil + I/R. Os animais do grupo remifentanil + I/R foram submetidos à infusão de remifentanil ($2 \mu\text{g kg}^{-1} \text{min}^{-1}$) por 60 min, metade dos quais iniciou antes da indução da isquemia. Coletando os tecidos do íleo, a avaliação dos danos foi baseada nas respostas contráteis ao carbacol, nos níveis de peroxidação lipídica e infiltração de neutrófilos e na observação das características histopatológicas no tecido intestinal.

Resultados: Após a reperfusão, uma diminuição significativa da resposta contrátil induzida por carbacol, um notável aumento tanto da peroxidação lipídica quanto da infiltração de neutrófilos e uma lesão significativa da mucosa foram observados. A média da resposta contrátil no grupo remifentanil + I/R foi significativamente diferente daquela do grupo I/R. A peroxidação lipídica e a infiltração de neutrófilos também foram significativamente suprimidas pelo tratamento. As amostras de tecido do grupo I/R apresentaram grau 4 na avaliação histopatológica. No grupo remifentanil + I/R, por outro lado, a lesão da mucosa foi moderada, apresentando estadiamento de grau 1.

Conclusões: O pré-tratamento com remifentanil pode atenuar a lesão intestinal de I/R em um grau notável, possivelmente pela redução da peroxidação lipídica e da infiltração leucocitária. © 2013 Sociedade Brasileira de Anestesiologia. Publicado por Elsevier Editora Ltda. Todos os direitos reservados.

Introduction

Activated opioid receptors (ORs) protects against ischemia/reperfusion (I/R) injury in heart and neurons.¹⁻³ Opioid peptides and ORs are present in gut.^{4,5} An ultra-short-acting phenylpiperidine opioid analgesic, remifentanil is cardioprotective against the I/R injury via mediating ORs,^{1,6} activating protein kinase C (PKC), and driving mitochondrial ATP-sensitive potassium (KATP) channels⁷ which mimic ischemic preconditioning (PC).^{1,8}

Sepsis, hemorrhage, intestinal transplantation, severe burns, and mesenteric thrombosis cause mesenteric ischemia,^{9,10} making a dramatic impact on the intestine. Intestinal I/R have a major role in the development of primary graft dysfunction.^{11,12} The intestinal tissue is very sensitive to the I/R. In fact, even though the gastrointestinal tract represents about 5% of the body weight, its oxygen consumption constitutes roughly 20% of the total oxygen usage.¹³ Interruption of the oxygen supply decreases the intracellular ATP content, disturbing the cellular homeostasis through the increased reactive oxygen species (ROS). Reperfusion induces structural and functional damage of mucosa and interstitial edema (reperfusion injury),¹⁴ which is primarily due to the generation of ROS from various sources including the electron transport chains of mitochondria, xanthine oxidase metabolism, prostaglandins, endothelial cells, and activated neutrophils.¹⁴⁻¹⁷ I/R damages the mucosal barrier function, causing the mucosal and vascular permeabilities and bacterial translocation, ending up with

systemic inflammation and multiple-organ failure.¹⁸ It also changes the contractile response of the intestinal smooth muscle,¹⁹⁻²² which is associated with the local inflammatory response augmented by the infiltrating neutrophils¹⁰ and ROS.²³

Some pharmacological agents including anesthetics produce PC.²⁴⁻²⁸ Specifically, ORs are involved in the PC of rat heart.^{7,8} Moreover, pharmacologic preconditioning induced by remifentanil also protects against the I/R-induced cardiac injury.³ On the other hand, whether or not the possible beneficial effect occurs in the intestinal I/R injury is extremely limited. Only one study by Cho et al.²⁹ reports that remifentanil protects the intestine against I/R injury. They clearly demonstrate that remifentanil significantly reduces oxidative stress, inflammatory response, and tissue injury. However, they did not study directly the effect on physiological functions of the tissue (e.g. contractility). Therefore, our aim was to determine whether remifentanil has beneficial effect on the I/R-disturbed intestinal contractility. We evaluated the contractile response, histopathological changes and measured the tissue levels of malondialdehyde (MDA), lipid peroxidation marker, and myeloperoxidase, neutrophil infiltration marker, (MPO).

Materials and methods**Animals**

A total of 32 Wistar adult male rats weighing 220 ± 20 g were used in the study. The animals were obtained from the

Bulent Ecevit University Animal Care Unit and housed under standard conditions. They had free access to commercial chow and water. On the day before the surgical procedures, the animals were fasted overnight but allowed ad libitum access to water. They were maintained in their cages at a constant room temperature using a 12 h:12 h light/dark cycle. All of the procedures described here were approved by the local animal ethics committee of the institution.

Operative procedures

Rats were anesthetized with sodium thiopental intraperitoneally (60 mg kg^{-1}) followed by conducting laparotomy through a midline incision into the peritoneal cavity. After the small bowel was exteriorized gently to the left onto moist gauze, animals were subjected to 30 min of ischemia by ligation of the superior mesenteric artery (SMA), using 3/0 silk thread. Intestinal ischemia was confirmed by the lack of pulse in the mesenteric artery and the pale color of the jejunum and ileum. Afterward, the intestines were returned to the abdomen, which was closed with 2 small clamps. At the end of 30 min of ischemia, the abdomen was reopened and the thread was gently removed to allow reperfusion of the blood flow, which was confirmed by observing the pulsation of the artery and its branches on the intestine. Body temperature was maintained at 37.8°C by heating lamp during the procedure of I/R.

Experimental groups

Animals were divided into 4 groups each of 8 animals: (a) Sham control group, animals underwent laparotomy without performing the occlusion of SMA; (b) Sham plus remifentanil (remifentanil alone) group, rats were treated with remifentanil and a sham operation without SMA occlusion; (c) I/R control group, the SMA was occluded for 30 min followed by 3 h of reperfusion; (d) I/R plus remifentanil group, rats subjected to I/R were treated with remifentanil.

Remifentanil (Glaxo Group Limited, Lake Forest, IL, USA) pretreatment was carried out by infusion ($2 \mu\text{g kg}^{-1} \text{ min}^{-1}$) via tail vein for 30 min prior to the occlusion of SMA and for additional 30 min during the whole period of ischemia. Catheterization of the tail vein was carried out with a 24-g Polyneo catheter (Polymed, Poly Medicure Ltd., Haryana, India) for administration of the infusion. Animals in the sham control and the I/R groups were instead given sterile serum physiological solution in the same volume. Following the reperfusion period, the animals were killed by administration of anesthetic at lethal doses; thereafter, samples of ileum were collected and processed for each experimental protocol.

Ileal longitudinal muscle contractility

The ileal longitudinal muscle contractile activity was evaluated in isolated ileal segments after 3 h of reperfusion in an organ bath. Strips of longitudinal muscle at 5-mm length were removed 1 cm away from the ileocecal junction. After removing the first strip for histological evaluation, second, third, and fourth samples at the same size were collected

for contractility and biochemical analyses. Strips were longitudinally suspended under 2-g load in 20 mL organ bath filled with Krebs solution (in mmol L^{-1} : NaCl, 118.5; KCl, 4.8; KH_2PO_4 , 1.2; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.2; CaCl_2 1.9; NaHCO_3 , 25; glucose, 10.1). The solution was gassed with a mixture of 5% CO_2 and 95% O_2 and maintained at 37°C . After 60 min equilibration with 2 g load, carbachol was added to the organ bath at final various concentrations ranging from 10^{-7} to $10^{-2} \text{ mol L}^{-1}$. Active force development was recorded at each concentration to determine the dose-response relationship. Isometric force was monitored by external force displacement transducer (FDA-10A, Commat Co, Ankara, Turkey) using MP 30 software (MP30 Biopac Systems Inc, Santa Barbara, CA, USA).

Histopathologic scoring of the ischemia–reperfusion injury to small intestine

The segments of ileum were fixed in 10% formalin and embedded in paraffin. Intestinal paraffin sections were stained with hematoxylin and eosin for morphological analysis. Histopathological evaluation of the reperfused-intestinal tissue was based on a staging method described by Hierholzer et al.³⁰ and the evaluation was graded from 0 to 4. In grade 0, no specific pathological changes are observed: normal structure of gut wall, including villi, crypts, lamina propria, and muscularis externa. In grade 1, mild mucosal damage is assessed: denudation of villi epithelium, otherwise normal structure. In grade 2, moderate damage occurs: loss of villus length and epithelial sloughing with evidence of congestion, hemorrhage, and inflammation in the mucosa, but no change in submucosa or muscularis externa. In grade 3, extensive damage is observed: loss of a large number of villi including denudation, sloughing, and the presence of granulomatous tissue with the damage localized to submucosa and muscularis. In grade 4, there is severe damage and necrosis: inflammation and necrosis in areas throughout the thickness of the intestinal wall. The investigator who performed these measurements was unaware of the experimental design.

Determination of tissue MDA activity

Intestinal lipid peroxide levels were measured by a method described by Casini et al.³¹ Briefly, by using a motor-driven pestle, tissue samples were homogenized in ice-cold TCA by adding 10 mL of 10% TCA per gram of tissue. Following centrifugation, 750 μL of supernatant was added to equal volume of 0.67% thiobarbituric acid and heated to 100°C for 15 min. The absorbance of the samples was then measured spectrophotometrically at 535 nm.

Determination of tissue MPO activity

The activity of MPO within the supernatant of the homogenate was determined from the H_2O_2 -dependent oxidation of *o*-dianisidine.³² Aliquots of supernatant (0.1 mL) were added to 2.9 mL of reaction mixture containing 0.167 mg mL^{-1} of *o*-dianisidine, and 20 mM of H_2O_2 solution, which were prepared in 50 mM of PB. Immediately after

adding the aliquot to the mixture, the change in absorbance at 460 nm was measured for 5 min. One unit of MPO activity was defined as that degrading 1 μmol of peroxide per min at 25 °C. The activity was then normalized as unit per mg of tissue (U mg^{-1}).

Data analysis

Values for the experiments dealing with contractility were normalized for per g of tissue. Each data point represents mean \pm S.E.M. For statistical evaluation, SPSS 11.0 statistical software package program was used (SPSS Inc., Chicago, IL, USA). Non-parametric tests were performed since each group consisted of eight replicate samples. Thus, using Kruskal–Wallis variance analysis (ANOVA), all groups were compared in terms of existence of heterogeneity. Once statistically meaningful heterogeneity was detected among all groups, individual groups were compared with each other by employing Tukey's test. p Values of less than 0.05 were considered significant.

Results

Ileal longitudinal muscle contractility

Carbachol caused a dose-dependent contractile effect on the terminal ileum segments from all groups, providing sigmoid curves with their maximal responses (E_{max} values) (Fig. 1). The E_{max} value for carbachol was significantly lower in the I/R control than in the sham group (12.83 ± 0.76 vs. 22.19 ± 2.10 g/g tissue, respectively). In other words, the contraction in response to carbachol was significantly and dose-dependently reduced by the induction of I/R, as indicated by a downward shift of the curve. The statistical difference between these groups was significant at all doses

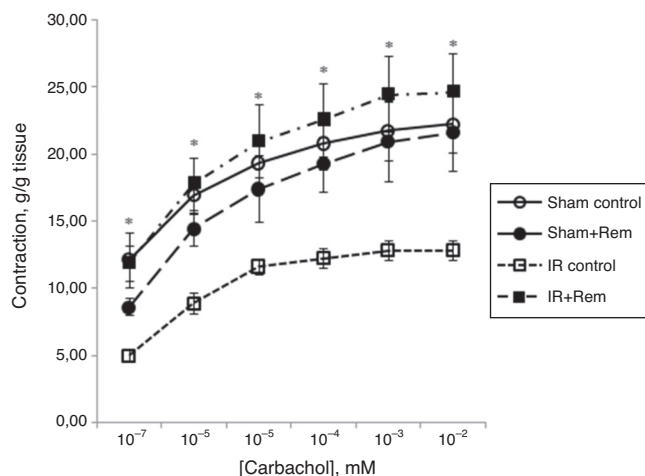


Figure 1 Concentration–response curves of carbachol in longitudinal ileum muscle isolated from the sham control, sham + Rem, IR control, and IR + Rem. Each data point is the mean \pm S.E.M. of eight experiments. * $p < 0.05$ indicates statistically meaningful difference from the IR control group (IR control vs all other three groups at each concentration of carbachol). (Rem; remifentaniol, IR; ischemia–reperfusion).

of carbachol ($p < 0.01$). Reduced contractility was significantly ameliorated by the treatment of remifentaniol. This effect was statistically significant at each dose of carbachol, compared to the I/R group. As seen in Fig. 1, contraction responses of sham group, remifentaniol alone group, and remifentaniol + I/R group, however, provided a similar curves at all carbachol doses, being statistically indifferent from each other ($p > 0.05$). Comparing E_{max} values, the I/R group (12.83 ± 0.76 g/g tissue) was only about 58% of the sham group (22.19 ± 2.10 g/g tissue). Average E_{max} value of the sham group was not statistically different from those of the both remifentaniol alone (21.55 ± 2.85 g/g tissue) and remifentaniol + I/R (24.60 ± 2.86 g/g tissue) groups.

Histopathological findings

Analyzing six sections per group, the most dramatic alterations were observed in the I/R group (Fig. 2C; Table 1). Inflammation, mucosal denudation, and edema were clearly detected on some parts of the mucosa and submucosa in addition to necrotic areas throughout the intestinal wall. Sloughing on some of the surface epithelium was also obvious. The I/R group was, therefore, evaluated as grade 4. In the sham group, as demonstrated in Fig. 2A, normal structure of gut wall with villi, crypts, lamina propria, and muscularis layer was clearly observed. The group was given a grade 0. Remifentaniol alone group has provided very similar appearances to those observed in the sham group; namely, no specific pathological changes were observed; thus, it was graded as 0 (Fig. 2B). Remifentaniol + I/R group has moderate mucosal damage with only minimal focal denudations of the villi epithelium; therefore, the group received a grade 1 (Fig. 2D).

MDA levels

The MDA content of the sham group animals averaged 73.29 ± 7.46 nmol g^{-1} tissue, while animals subjected only to the I/R were found to have 156.82 ± 15.74 nmol g^{-1} tissue (Fig. 3), a significant increase in MDA content ($p < 0.001$). Pretreatment with remifentaniol (remifentaniol + I/R group) significantly reduced the I/R-induced intestinal MDA content to the sham group levels. With an average value of 107.80 ± 8.93 nmol g^{-1} tissue, this group was statistically indistinguishable from the sham group ($p = 0.143$), but statistically different from the I/R group ($p = 0.02$). On the other hand, the average MDA content in the remifentaniol alone

Table 1 Semi-quantitative histological grading of cross-sections of the rat ileum (6 sections/group).

	Mean \pm S.E.M.
Sham control	0
Sham + Remifentaniol	0
I/R control	3.83 ± 0.16
I/R + Remifentaniol	1.17 ± 0.16^a

Analyzing with one-way ANOVA, statistical significance among two groups.

^a $p = 0.00005$.

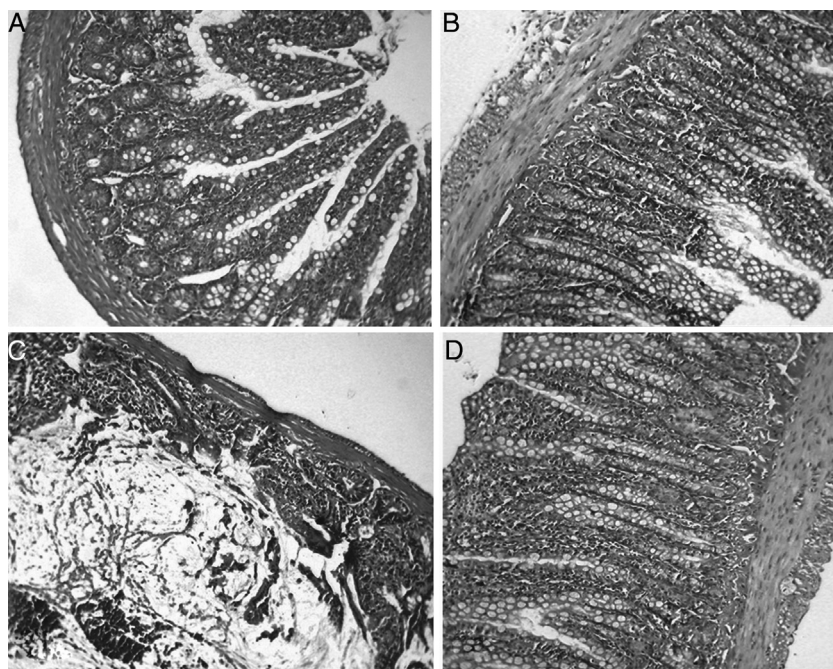


Figure 2 Light micrographs of the rat intestinal tissue: (A) Sham-operated control group; normal architecture of intestinal wall with no any pathological alteration (grade 0); (B) Sham + Rem; normal histomorphologic appearance of intestinal tissue (grade 0); (C) I/R control; the most extensive morphological alterations detected, such as ulcerated intestinal lumen, significantly decreased muscular layer, loss of epithelium and glands, and having superficial exuda and mucoid material (grade 4); (D) IR + Rem; the pretreatment improves histopathological alterations observed after the I/R; inflamed intestinal tissue with moderate mucosal thinning, patchy loss of epithelium, and mix type inflammatory cell reaction which is dominated by polymorph nuclear leukocytes (grade 1). (H&E stain $\times 200$, Scale bar = $50 \mu\text{m}$). (Rem; remifentanil, I/R; ischemia-reperfusion).

group ($84.03 \pm 14.90 \text{ nmol g}^{-1} \text{ tissue}$) was not statistically different from that in the sham group ($p = 0.946$).

MPO activity

The average MPO enzyme activities of the tissues from animals subjected to sham operation, remifentanil alone, I/R, and remifentanil + I/R were (in $\text{U mg}^{-1} \text{ tissue}$) 0.63 ± 0.17 ,

0.54 ± 0.08 , 1.60 ± 0.28 , and 0.83 ± 0.14 , respectively (Fig. 4).

The I/R caused approximately a 2.5-fold increase in the MPO activity of the tissue compared to the basal level of the activity ($p = 0.004$). Pretreatment with remifentanil completely abolished the increased enzymatic activity resulting from the I/R insult; the MPO activity of samples from animals pretreated with remifentanil was significantly different from that in the I/R group ($p = 0.049$). No statis-

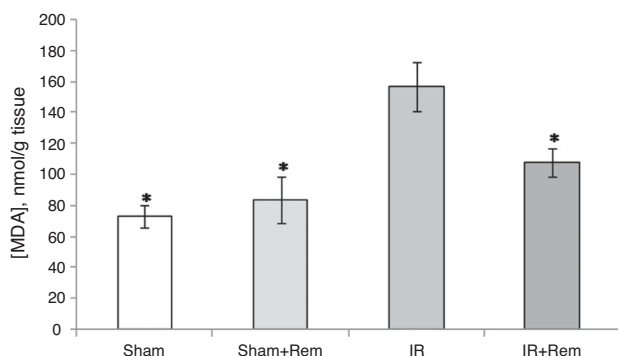


Figure 3 Average malondialdehyde (MDA) content of ileum samples from the sham control, sham + Rem control, IR control, and IR + Rem. Data are expressed as mean \pm S.E.M. ($n = 8$). Compared to the IR control group, statistically significant difference is denoted as $*$ ($p < 0.05$). (Rem; remifentanil, IR; ischemia-reperfusion).

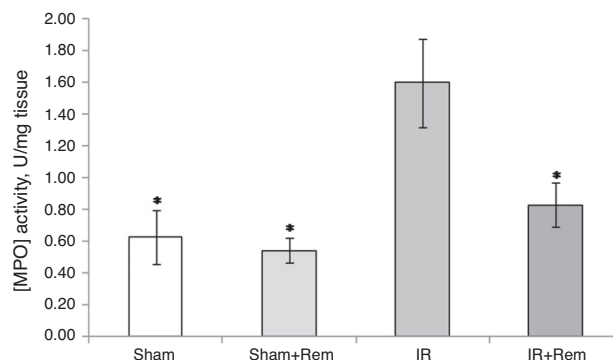


Figure 4 Average myeloperoxidase (MPO) activity of the ileum samples collected from the sham control, sham + Rem control, I/R control, and IR + Rem. Data are presented as mean \pm S.E.M. of eight animals in each group. Compared to the IR control group, statistically significant difference is denoted as $*$ ($p < 0.05$).

tical difference was observed in the MPO activity between the sham-group and the remifentanil alone group.

Discussion

The present study demonstrates that remifentanil may have strong protective potential against the intestinal I/R injury. Evidences indicate that remifentanil attenuates the I/R-induced: (1) intestinal pathological changes; (2) decrease in contractile response; (3) increased neutrophil infiltration; and (4) elevated lipid peroxidation.

There are many published studies that show beneficial effects of pharmacological PC with remifentanil on reperfusion injury of various tissues. However, those concerned with pharmacological PC of the intestine with remifentanil are very limited and lacking functional aspects, such as motility and secretion. In fact, only one study has been dealing with this question. Cho et al.²⁹ have shown that remifentanil attenuates intestinal I/R injury in mice by reducing lipid peroxidation and systemic inflammation. Our data confirmed the outcomes of this study in regard to the inhibition of oxidative stress and inflammation and demonstrated the direct involvement of leukocytes in tissues. However, the study did not directly investigate the effect of remifentanil on physiological functions of the tissue, such as motility and/or secretion. Therefore, the current study takes a further step, showing that the pretreatment with remifentanil ameliorates the reduced contractility due to I/R—a novel finding potentially linked to the anti-inflammatory and antioxidant activity of remifentanil.

Intestinal I/R may cause multiple organ failure, severe morbidity, and even death.^{1,16} Both acute and chronic ischemia make a substantial impact on gut functionally as well as structurally, leading to dismotility, abnormal absorption, and altered barrier function against bacterial translocation.^{10,14,33} Acute intestinal I/R causes such long-term structural alterations as the development of hypertrophy, cellular dysfunction, neuronal death, necrosis within the myenteric plexus,^{19,22,33} and the infiltration of inflammatory cells (i.e., neutrophils).^{30,34} Many published studies demonstrate deleterious effects of I/R on intestinal motility, such as delayed gastrointestinal transit,^{35–37} altered migrating motor complex,³⁸ and impaired motor response to any stimulation.^{20,39,40} Inflammatory response within the muscular cells and decrease in contractility of the circular smooth muscle are triggered by I/R.^{30,34} Integrity of the enteric neuronal network is necessary for normal gastrointestinal motility. Therefore, the I/R may disturb the motor function of the gut by altering the properties of enteric neurons or the neuro-effector transmission.²² These changes are probably the outcome of the inflammatory response induced by the I/R injury. The intestinal damage we observed may be associated with the inflammation, which is marked specifically with an increased amount of neutrophils in the tissue. Inhibiting the activation and the endothelial adherence of neutrophils reduce the I/R-induced damage to the mucosa.⁴¹ In response to the inflammation of the muscle, neutrophils are activated, contributing possibly to the muscle damage. Therefore, the motility disturbance occurring after the I/R was more likely resulted from the severe muscle damage and associated inflammatory reaction that

we observed. The treatment with remifentanil reduced not only the lipid peroxidation in the tissue but also the leukocyte infiltration, tissue damage, and disturbed contractile response.

Returning of the oxygenated blood to the ischemic intestine further exacerbates the tissue damage mainly because of the increased generation of ROS, causing oxidative stress.^{17,42} Primary sources of ROS during the reperfusion are xanthine oxidase and activated neutrophils releasing the pro-inflammatory mediators and cytotoxic substances, such as MPO.¹⁷ Intestinal reperfusion injury is regarded as an inflammatory disease characterized with the recruitment of leukocytes.⁹ ROS liberated in reperfusion further stimulate inflammatory response, enhancing the recruitment and activation of neutrophils. The tissue MPO activity, proportional to the amount of accumulated-neutrophils, mirrors the mucosal injury and the rate of neutrophil infiltration in postischemic tissue.^{17,43} Hence, following the I/R, the increased MPO activity in the tissue indicated the enhanced-inflammatory response in the current study. The elevated histologic scoring of the tissue lesions and the decreased contractile response coincided with the significant elevation of both MPO activity and lipid peroxidation of the tissue in I/R group. One of the deleterious consequences of oxidative damage is lipid peroxidation, which eventually causes structural and functional disturbances to the membranes. Significant reduction in both MPO activity and MDA content were observed in the remifentanil + I/R group which indicates a significant limitation to the inflammation. Moreover, contractile response to carbachol in the same group appeared to be maintained very close to that in the sham control group. These findings were supported by the histopathological observation that remifentanil successfully rescued the integrity of the tissue from I/R injury.

The opioid-induced PC protects against the cardiac I/R injury. Specifically, PC by remifentanil, an ultra-short-acting phenylpiperidine opioid analgesic, confirms the cardio-protection.³ Activation of κ - and δ -ORs was involved in PKC activation, opening of mitochondrial KATP channel, and increasing anti-apoptotic protein expression, important targets of cardio-protection in ischemic and pharmacologic PC.^{7,8} The toll-like receptor signaling in inflammatory pathway is also associated with the opioid-induced protection in brain and cell cultures. Following activation of the toll-like receptor 2, opioid agonists have an inhibitory effect on proinflammatory cytokine production in human monocytes. The inhibition is mediated exclusively by μ -ORs.² In the current study, we recognized that the integrity of intestinal mucosa and contractile response of the tissue remained nearly normal in remifentanil + I/R group. Our data demonstrated that remifentanil decreased both lipid peroxidation and neutrophil accumulation in ischemic tissue which may be accounted for underlying mechanism of above mentioned findings. However, it'd have been better and confirmative to evaluate the effects of an antagonist/agonist of μ -ORs on the present model besides examining a dose-response pattern of remifentanil.

Cells in the gastrointestinal tract form a site in which the ORs exist at high levels. The presence of ORs along the tract is demonstrated in nerve and smooth muscle.⁴ Many studies report that the protection by remifentanil is mediated by

ORs.^{44–46} On the other hand, some suggest the mediation of OR-independent mechanism.² However, Zhang et al.⁵ show that the endogenous opioid peptides might play a major role in ischemic PC of the small intestine. In model of croton oil-induced inflammation of mice intestine, expression of the μ -OR is reported to be up-regulated in the myenteric plexus of jejunum. Similarly, patients with IBD have an increased expression of μ -OR mRNA in their inflamed small bowel and colon in respect to the healthy intestinal tissue.⁴⁷ In the present study, we do not know whether the protective effect of remifentanil is mediated through OR-dependent mechanisms or not. However, it is tempting to speculate that the beneficial effect is due to the OR-mediated processes since the tissue has an abundant amount of ORs and since the inflammatory insults induce the up-regulation of the ORs. If this is the case, then the pretreatment with remifentanil possibly mimics the effect of ischemic PC. Namely, acting via ORs, remifentanil may eventually activate PKC through the turnover of phosphatidylinositol induced by the activation of the membrane-bound phospholipase C or D. PKC then phosphorylates and stabilizes the open state of both the mitochondrial and the sarcolemmal KATP channels which ultimately elicits cytoprotection as observed in remifentanil-induced cardioprotection.⁷ The pretreatment with remifentanil may also have an OR-mediated inhibitory effect on the generation of proinflammatory cytokines by monocytes in response to the stimulation of toll-like receptor 2.² In these cases, the administration of an OR antagonist (i.e. Naloxone) would have been detrimental to the preventive effects observed in the present study. On the other hand, if the mechanisms are non-receptor-mediated, the remifentanil-induced intestinal protection may be multifactorial. In this case, as reported on remifentanil PC of hepatic I/R, inducible nitric oxide synthase (iNOS) may mediate the preventive effects in intestinal tissue. For instance, both lipid peroxidation and inflammation could be attenuated by an elevated generation of the endogenous nitric oxide (NO) which would limit the amount of ROS and attenuate the inflammatory response. It is well documented that the endogenous NO may exhaust ROS and neutrophil-mediated tissue injury; hence, reducing inflammatory response significantly. It is also shown that the cellular damage induced by the I/R is involved with a significant decrease of NO. Therefore, remifentanil may be associated with the induction of iNOS expression and consequent generation of the endogenous NO in intestinal tissue challenging to the reperfusion injury. If so, the protective effects would have been blocked by a NOS inhibitor but not with Naloxone.²

In conclusion, we have demonstrated that the treatment of rats with remifentanil prior to the I/R provided protection against I/R-induced mucosal damage and contractile disturbance. Possible mechanism responsible for these salutary activities of remifentanil may probably be multifactorial. However, as the present study has given evidences, the attenuation of neutrophil infiltration and the reduction of oxidative stress appear to be of primary importance to get the beneficial effects. Further studies, which will focus on the mechanisms of protective effects of remifentanil in depth, are required for sure. In conclusion, we have shown that the administration of remifentanil during ischemia could efficiently be protective against the most histological, cytological, biochemical, and physiological aspects of

intestinal injury, providing therapeutic potential for clinical application in intestinal ischemia and ischemia-related disorders. Moreover, the current study implicates that the application of remifentanil as analgesic agent may be preferred for those suffering from intestinal hypoperfusion and undergoing anesthesia.

Conflicts of interest

The authors declare no conflicts of interest.

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