Propofol attenuates intestinal mucosa injury induced by intestinal ischemia-reperfusion in the rat

[Le propofol atténue les lésions de la muqueuse intestinale provoquées par l'ischémie-reperfusion intestinale chez le rat]

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Purpose: We investigated whether propofol at a sedative dose can prevent intestinal mucosa ischemia/reperfusion (I/R) injury, and if propofol can attenuate oxidative stress and increases in nitric oxide (NO) and endothelin-1 (ET-1) release that may occur during intestinal I/R injury.

Methods: Rats were randomly allocated into one of five groups (n = 10 each): (i) sham control; (ii) injury (one hour superior mesenteric artery occlusion followed by three hours reperfusion); (iii) propofol pre-treatment, with propofol given 30 min before inducing intestinal ischemia; (iv) simultaneous propofol treatment, with propofol given 30 min before intestinal reperfusion was started; (v) propofol post-treatment, with propofol given 30 min after intestinal reperfusion was initiated. In the treatment groups, propofol 50 $mg \cdot kg^{-1}$ was administrated intraperitoneally. Animals in the control and untreated injury groups received equal volumes of intralipid (the vehicle solution of propofol) intraperitoneally. Intestinal mucosa histology was analyzed by Chiu's scoring assessment. Levels of lactic acid (LD), NO, ET-1, lipid peroxidation product malondialdehyde (MDA) and superoxide dismutase (SOD) activity in intestinal mucosa were determined.

Results: Histological results showed severe damage in the intestinal mucosa of the injury group accompanied by increases in MDA, NO and ET-1 and a decrease in SOD activity. Propofol treatments, especially pre-treatment, significantly reduced Chiu's scores and levels of MDA, NO, ET-1 and LD, while restoring SOD activity.

Conclusion: These findings indicate that propofol attenuates intestinal I/R-induced mucosal injury in an animal model. The response may be attributable to propofol's antioxidant properties, and the effects of inhibiting over-production of NO and in decreasing ET-1 levels.

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***Objectif** : Nous avons cherché à savoir si le propofol, en dose sédative, pouvait empêcher les lésions d'ischémie/reperfusion (I/R) de la muqueuse intestinale, et s'il pouvait atténuer le stress oxydatif et les augmentations dans la libération d'oxyde nitrique (NO) et d'endothéline-1 (ET-1) pouvant survenir lors de lésions I/R intestinales.*

***Méthode** : Des rats ont été randomisés en cinq groupes (n = 10 chacun) : (i) faux témoin (sham control) ; (ii) lésion (occlusion de l'artère mésentérique supérieure d'une heure suivie par reperfusion de trois heures) ; (iii) prétraitement au propofol, avec administration de propofol 30 min avant de provoquer l'ischémie intestinale ; (iv) traitement simultané au propofol, avec administration de propofol 30 min avant le début de la reperfusion intestinale ; (v) traitement ultérieur au propofol, avec administration de propofol 30 min après le début de la reperfusion intestinale. Dans les groupes de traitement, du propofol a été administré en dose intrapéritonéale de 50 $mg \cdot kg^{-1}$. Les animaux des groupes témoin et lésions non-traitées ont reçu des volumes équivalents d'Intralipid (la solution véhicule du propofol) en dose intrapéritonéale. L'histologie de la muqueuse intestinale a été analysée par l'évaluation des points de Chiu (Chiu's scoring assessment). Les niveaux d'acide lactique*

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(LD), NO, ET-1, l'activité de produits de péroxydation lipidique de malondialdéhyde (MDA) et de superoxyde dismutase (SOD) dans la muqueuse intestinale ont été déterminés.

***Résultats** : Les résultats histologiques ont montré des lésions graves de la muqueuse intestinale dans le groupe lésions, accompagnées d'une augmentation de MDA, NO et ET-1 et une diminution de l'activité SOD. Les traitements au propofol, particulièrement le prétraitement, ont réduit de façon significative les résultats et niveaux de MDA, NO, ET-1 et LD sur l'échelle de Chiu, tout en restaurant l'activité SOD.*

***Conclusion** : Ces résultats indiquent que le propofol atténue les lésions de la muqueuse intestinale provoquée par I/R chez le modèle animal. La réaction peut être attribuée aux propriétés anti-oxydantes du propofol, ainsi qu'aux effets d'inhibition de la surproduction de NO et de la diminution des niveaux de ET-1.*

INTESTINAL ischemia/reperfusion (I/R) injury is a serious condition which may result from hemorrhagic, traumatic or septic shock, or severe burns, and certain surgical procedures including small bowel transplantation, abdominal aortic surgery and cardiopulmonary bypass (CPB).[1] It is well known that intestinal I/R not only leads to the injury of intestine itself, but may also cause multiple organ dysfunction owing to damage of the intestinal mucosal barrier.[2] Of particular interest, compromised peripheral perfusion during CPB and the resulting gastrointestinal mucosal injury have been shown to lead to decreased mucosal barrier function, which may allow translocation of intestinal flora and endotoxemia and subsequently increased systemic inflammation.[3,4] This may lead to and/or further enhance oxidative stress during CPB and result in more eventful postoperative myocardial functional recovery.

The mechanisms of intestinal mucosa injury after intestinal I/R are complex. Reactive oxygen species (ROS)-induced lipid peroxidation is known to be one of the major factors causing intestinal I/R injury, and the administration of free radical scavengers appears to prevent intestinal mucosa from intestinal I/R injury.[5] We have recently shown that antioxidant intervention during cardiac surgery under CPB attenuated gastric and intestinal mucosa injury and resulted in ameliorated postoperative myocardial injury,[6] suggesting that antioxidant intervention can attenuate intestinal I/R injury in the clinical setting. Propofol is an *iv* anesthetic with antioxidant properties[7,8] that is commonly used during cardiac surgery and postoperative sedation.[9–11] Propofol has been shown to enhance tissue antioxidant capacity in various tissues in a rat model.[12] Interestingly, low-dose propofol sedation attenuates the formation of ROS in tourniquet-induced ischemia-reperfusion injury in humans.[13] It is unknown, however, whether propofol at a sedative dose can attenuate intestinal I/R-induced increase in oxidant stress and intestinal mucosal injury.

It has been reported that over-production of nitric oxide (NO) in intestinal mucosa tissue following intestinal I/R can aggravate lipid oxidative damage[14,15] and that an increase of endothelin-1 (ET-1) is involved in the pathogenesis of intestinal I/R-induced intestinal mucosal injury.[16,17] Therefore, the current study was undertaken to clarify whether propofol can prevent intestinal mucosa I/R injury, and to investigate its effects on NO, ET-1 release during intestinal I/R, in an in *vivo* rat model. The accumulation of lactic acid (LD), a product from glucose metabolism in anaerobic metabolism, was used as an indirect index of intestinal ischemia.

Methods

Animal model

The current study was approved by the Animal Care Committee of Sun Yat-sen University, China and was performed in accordance with National Institutes of Health guidelines for the use of experimental animals. Fifty adult pathogen-free male Wistar rats weighing between 230 and 302 g, were housed in individual cages in a temperature-controlled room with alternating 12 hr light/dark cycles, and acclimated for one week before the study. Food was removed eight hours prior to the study, but all animals had free access to water.

All animals were anesthetized with pentobarbital (30 $mg \cdot kg^{-1}$ body weight, intraperitoneally), and the small intestine was exteriorized by midline laparotomy. The intestinal I/R injury was established by occluding the superior mesenteric artery (SMA) with a microvessel clip for 60 min followed by 180 min reperfusion, as reported by Mitsuoka *et al.*[18] Ischemia was recognized by the existence of pulseless or pale colour of the small intestine. The return of pulses and the re-establishment of the pink colour were assumed to indicate valid reperfusion of the intestine.

Experimental protocol

The rats were randomly allocated into one of the five groups ($n = 10$ per group): (i) control group (Control), in which sham surgical preparation including isolation of the SMA without occlusion was performed; (ii) injury group (Injury), in which intestinal I/R was produced by clamping SMA for one hour followed by declamping (i.e., reperfusion) for three hours; (iii)

Propofol pre-treatment group (Pre-Prop), in which propofol was given 30 min before intestinal ischemia was induced; (iv) simultaneous propofol treatment group (Simu-Prop), in which propofol was given 30 min before intestinal reperfusion was started; (v) post-treatment group (Post-Prop), in which propofol was given 30 min after intestinal reperfusion was started. In the treatment groups, propofol (Diprivan, propofol 1%, CG411, AstraZeneca, Caponago, Italy) 50 $mg \cdot kg^{-1}$ was administrated intraperitoneally. Animals in the control and injury groups received an equal volume of intralipid (vehicle solution of propofol) by *ip* injection. The dose of propofol (i.e., 50 $mg \cdot kg^{-1}$ *ip*) was chosen based on a preliminary experiment. This experiment showed that propofol 50 $mg \cdot kg^{-1}$ *ip*), a dose which inhibits rat hippocampal acetylcholine release to a lesser extent than does propofol 100 $mg \cdot kg^{-1}$ *ip*,[19] produced a sedative response in rats, as determined by loss of reflex responses to a painful stimulus (needle skin prick), while remaining sensitive to skin incision. As propofol 60 $mg \cdot kg^{-1}$ *ip*, provides satisfactory anesthesia in rats,[20] we selected a slightly lower dose based on our preliminary study. Also, during the preliminary experiment, we found that neither intralipid nor physiological saline influenced the extent of intestinal mucosal damage in the injury group. Therefore, only intralipid was used as a vehicle control in the ensuing studies.

Preparation of specimens

After the completion of the experiments, the rats were killed with an *iv* overdose of pentobarbital sodium. A segment of 0.5–1.0 cm intestine was cut from 5 cm to terminal ileum, fixed in 4% formaldehyde polymerisatum, and embedded in paraffin for preparation. Another segment of small intestine was washed with cold saline and the intestinal mucosa was gently scraped off, dried with suction paper, and preserved at -70°C.

Histological measurement of intestinal mucosal injury

The segment of small intestine was stained with hematoxylin-eosin. Damage of intestinal mucosa was initially evaluated independently by two pathologists who were blinded to the study groups. The degree of injury was evaluated using a modified Chiu's method[21] according to changes of the villus and glands of the intestinal mucosa. The Chiu's score was graded as: 0, normal villus and gland; 1, changes at the top of villus and initial formation of subepidermal Gruenhagen's antrum; 2, formation of subepidermal Gruenhagen's antrum and slightly injured gland; 3, enlargement of subepidermal gap and engorgement of capillary vessel; 4, epidermis moderately isolated with lamina propria and injured gland; 5, top villus shedding; 6, obvious villus shedding and capillary vessel dilating; 7, lamina propria villus shedding, and distinct injured gland; 8, initially decomposed lamina propria; 9, hemorrhage and ulceration. A minimum of six randomly chosen fields from each rat were evaluated and averaged to determine mucosal damage.

Detection of lipid peroxidation and superoxide dismutase activity in intestinal mucosa

Intestinal mucosal tissues were homogenized on ice with normal saline, frozen in a refrigerator at -20°C for five minutes and centrifuged for 15 min at 4000 g. Supernatants were transferred into fresh tubes for the evaluation. The lipid peroxidation product malonedialdehyde (MDA) was measured by chemical analysis (Assay kits was supplied by Nanjing Jiancheng Biological Product, Nanjing, China) as previously described.[22,23] The results were calculated as $nmol \cdot 100\ mg^{-1}$ tissue. Superoxide dismutase (SOD) activity was evaluated by inhibition of nitroblue tetrazolium reduction by superoxide anion generated by the xanthine/xanthine oxidase system using a commercial assay kit (Nanjing Jiancheng Biological Product, Nanjing, China) as described.[22,23] The results were expressed as $U \cdot 100\ mg^{-1}$ protein.

Detection of NO level in intestinal mucosa

Intestinal mucosal tissues (100 mg) were weighed and made into 10% homogenate with 0.9 mL physiological saline. After centrifugation for ten minutes at 10000 g, the supernatant was placed in boiling water for three minutes and then centrifuged for five minutes at 10000 g. Supernatant (0.1 mL) was taken for analysis using a commercial assay kit (Nanjing Jiancheng Biological Product, Nanjing, China). Nitrate and nitrite (NOx) were measured as oxidized stable end products of NO and the total nitrite level in the sample was determined according to the method described by Miranda *et al.*[24] Results were calculated as $\mu mol \cdot 100\ mg^{-1}$ protein.

Detection of ET-1 level in intestinal mucosa

Endothelin-1 level was measured by enzyme linked immunoassay (ELISA) techniques (assay kit was supplied by Beijing East Asian Radioimmunoassay Technology Institute, Beijing, China) as previously described.[23] Briefly, 100 mg intestinal mucosal tissue was boiled in 1 mL of a mixture of 1 M acetate and 20 mM hydrochloride for ten minutes at 100°C, and then centrifuged at 10000 g for ten minutes at 4°C. The supernatant was filtrated, lyophilized, and dissolved

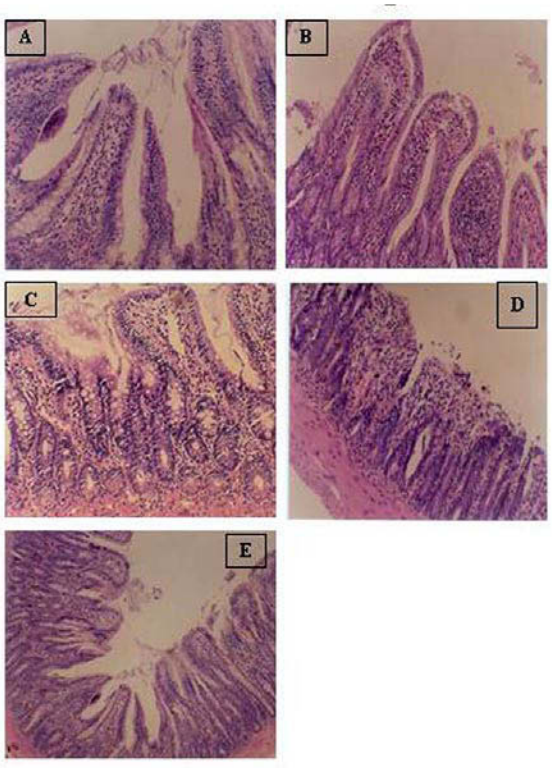

FIGURE 1 Histopathological changes of small intestine under light microscopy (× 200) A) In the control group, normal intestinal mucosa was seen. B) In the injury group, intestinal mucosa was damaged severely as shown by marked edema of mucosal villi and infiltration of necrotic epithelial and inflammatory cells. A large number of intestinal villi were severed and the gap between epithelial cells increased significantly. C) In the pre-treatment group, the damage was slight without significant edema. D) In the simultaneous treatment group, slight edema could be seen in intestinal villi, and some intestinal villi were severed. The gap between epithelial cells increased slightly. E) In the post-treatment group, a small number of intestinal villi were severed, and the increased gap between epithelial cells could be seen.

in 300 mL of buffer solution. This extracted peptide solution was applied to the ELISA plate. Endothelin-1 level in samples was determined using a standard curve generated from known concentrations of ET-1. All measurements were performed in triplicate, and the intra- and interassay variability were < 10%. Results were calculated as pg 100 mg^{-1} protein.

Detection of LD level in intestinal mucosa

Intestinal mucosal tissues were weighed and made into 10% homogenate. The LD content in tissues was determined using a chemical assay kit (Nanjing Jiancheng Biological Product, Nanjing, China) as described.[25] The results were expressed as mmol·g^{-1} protein.

Statistical analysis

Statistics were analyzed with SPSS 11.0 software (SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± SD. One-way analysis of variance was used for multiple comparisons and the least significant difference test was used for intra-group comparison. Correlation between different variables was assessed by Spearman's coefficient, and $P < 0.05$ was considered statistically significant.

Results

Histological changes of intestinal mucosa under light microscopy

As shown in Figure 1, in the control group, the villi and glands were normal and no inflammatory cell infiltration was observed in the mucosal epithelial layer (Figure 1A). In the injury group, severe edema of mucosal villi and infiltration of necrotic epithelial and inflammatory cells were observed, and intestinal glands showed evidence of mild injury. In addition, a large number of intestinal villi were severed, the gap of epithelial cells increased significantly, and blood and lymph vessels expanded markedly, indicative of severe mucosal damage (Figure 1B). In the pre-treatment group, no significant edema and necrotic mucosal villi were seen, indicating that the damage was very minimal (Figure 1C). In the simultaneous treatment group, slight edema could be seen in intestinal villi, and some intestinal villi were severed. Intestinal glands could be seen in some specimens, and the gap between epithelial cells increased slightly (Figure 1D). In the post-treatment group, a large number of intestinal villi were severed, and an increased gap between epithelial cells could be seen in severely damage areas, and blood and lymph vessels were expanded slightly (Figure 1E).

Evaluation of intestinal mucosal injury

As shown in the Table, Chiu's scores in the injury groups were significant higher than scores in the control group ($P < 0.01$). Compared with the injury group, Chiu's scores in the three treatment groups were significantly decreased (all $P < 0.01$), but all scores exceeded those observed in the control group ($P < 0.01$). Chiu's score in the pre-treatment group was markedly lower ($P < 0.01$) than values observed in the simultaneous and post-treatment groups, suggesting that pre-treatment with propofol is better than other treatment regimens.

TABLE Effects of propofol on SOD activity and the MDA, LD, NOx and ET-1 levels in intestinal mucosa

Groups	*Chiu's score*	*MDA* *(nmol·100 m^{-1})*	*SOD* *(U·100 m^{-1})*	*NOx* *(μmol·100 mg^{-1})*	*ET-1* *(mg Pg·100 mg^{-1})*	*LD* *(mmo·g^{-1})*
Control	0.98 ± 0.74	37.80 ± 5.10	40.82 ± 5.07	41.27 ± 8.60	284.12 ± 46.1	2.71 ± 0.65
Injury	9.15 ± 3.62*	82.76 ± 20.34*	24.75 ± 9.70*	84.36 ± 12.53*	691.50 ± 109.98*	4.21 ± 0.93*
Pre-Prop	3.67 ± 1.82*†	35.72 ± 9.24†	58.18 ± 6.94*†	51.24 ± 9.84†	278.43 ± 26.15†	2.99 ± 0.31†
Simu-Prop	5.75 ± 1.96*†‡‡	44.30 ± 18.35†	40.13 ± 7.62†‡	60.41 ± 15.89*†	357.51 ± 31.90**†‡‡	3.50 ± 0.36 **†
Post-Prop	5.98 ± 2.02*†‡‡	62.13 ± 16.67*†‡?	39.32 ± 8.43†‡	71.78 ± 17.23*†‡	421.32 ± 93.53 *†‡?	4.08 ± 1.04**‡

SOD = superoxide dismutase; MDA = malondialdehyde; LD = lactic acid; NOx = nitrate and nitrite; ET-1 = endothelin-1. Data are mean ± SD; $n = 10$. *$P < 0.01$, **$P < 0.05$ *vs* the control group; †$P < 0.01$ *vs* the injury group; ‡$P < 0.01$, ‡‡$P < 0.05$ vs the pre-treatment group (Pre-Prop); ?$P < 0.05$ *vs* the simultaneous group (Simu-Prop).

Changes of the MDA level and SOD activity in small intestinal mucosa

As shown in the Table, the MDA level in the injury group was significantly higher than that in the control group ($P < 0.01$). Compared with the injury group, MDA levels in the three treatment groups were markedly reduced ($P < 0.01$). However, the MDA level in the post-treatment group was significantly higher than observed in the pre-treatment and the simultaneous groups ($P < 0.01$ or 0.05, Table). In contrast, SOD activity in the injury group was significantly reduced ($P < 0.05$, injury *vs* control). Treatments with propofol markedly increased and restored SOD activity ($P < 0.01$, Pre-Prop, Simu-Prop or Post-Prop *vs* Injury, Table). Of interest, the SOD activity in the pre-treatment group was even higher than that observed in the control group ($P < 0.05$), and was also significantly higher than in other treatment groups ($P < 0.01$).

Changes of the NOx and ET-1 level in small intestinal mucosa

As shown in the Table, the NOx level in the injury group was greater than in the control group ($P < 0.01$). Nitric oxide levels in the three treatment groups were reduced as compared to the injury group ($P < 0.01$). The NO level in the pre-treatment group did not differ from that in the control group ($P > 0.05$, Pre-Prop *vs* Control, Table) but was markedly lower than that observed in the post-treatment group ($P < 0.01$). Similarly, the level of ET-1 in the injury group was increased as compared to the control group ($P < 0.01$). Compared with the injury group, the level of ET-1 was reduced by the treatments with propofol ($P < 0.01$). However, Pre-Prop, but not Simu-Prop or Post-Prop, restored ET-1 to the control value ($P > 0.05$, Pre-Prop *vs* Control). The ET-1 level in the Pre-Prop group was lower than that in Post-Prop group ($P < 0.01$).

Changes of the LD level in intestinal mucosa

The LD level in the injury and post-treatment groups were significantly higher than in the control group ($P < 0.05$). Pre-Prop and Simu-Prop, but not Post-Prop, significantly reduced the increase of LD as compared to the injury group ($P < 0.01$) (Table).

Correlation analysis

Overall ($n = 50$), strong positive correlations between Chiu's score and MDA ($r = 0.83$, $P < 0.0001$, Figure 2A), between MDA and ET-1 ($r = 0.89$, $P < 0.0001$, Figure 2C) and between Chiu's score and NOx ($r = 0.87$, $P < 0.0001$, Figure 2D) were identified. In contrast, ET-1 was inversely correlated to SOD activity ($r = -0.78$, $P < 0.0001$, Figure 2B). Also, strong positive correlations between MDA and NOx ($r = 0.83$, $P < 0.0001$, Figure 2E) as well as between NOx and ET-1 ($r = 0.77$, $P < 0.0001$, Figure 2F) were identified.

Discussion

We have demonstrated in a rat model that one hour occlusion of the SMA followed by three hours of reperfusion caused significant intestinal I/R injury as evidenced by pathological morphological changes and increased Chiu's scores seen in the intestinal mucosa, which is in accordance with a previous report.[5] The intestinal I/R injury was associated with dramatic increases in the intestinal mucosa, of MDA, ET-1, NO and LD, and a decrease in SOD activity. The novel finding of the current study is that propofol, at a sedative dosage, significantly attenuated SMA occlusion - reperfusion induced intestinal mucosal damage and the above mentioned biochemical changes when given either prior to, during SMA occlusion, or during the early phase of reperfusion. Most intriguingly, propofol pretreatment normalized changes of MDA, ET-1, NO and LD and stimulated an over-production

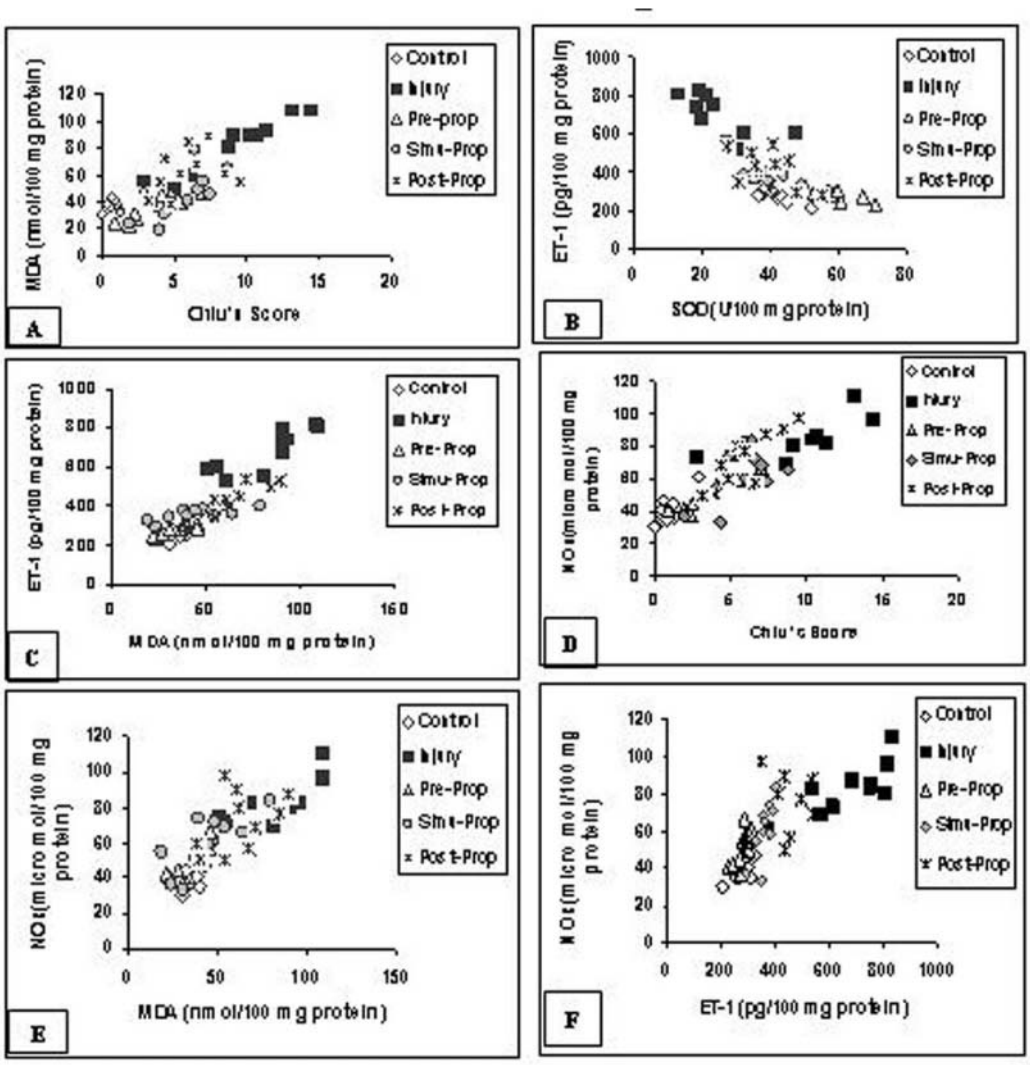

FIGURE 2 Correlations between Chiu's score and malonedialdehyde (MDA) (A), between endothelin (ET)-1 and superoxide dismutase (SOD) (B) or MDA (C), between Chiu's score and NOx production (D), between NOx production and MDA (E), and between NOx production and ET-1 (F). Strong positive correlations between Chiu's score and MDA (r = 0.83, 95% confidence interval: 0.71–0.90, $P < 0.0001$, A), between MDA and ET-1 (r = 0.89, 95% confidence interval: 0.81–0.94, $P < 0.0001$, C), between Chiu's score and NOx (r = 0.87, 95% confidence interval: 0.78–0.92, $P < 0.0001$, D), between NOx and MDA (r = 0.83, 95% confidence interval: 0.71–0.90, $P < 0.0001$, E), and between NOx and ET-1 (r = 0.77, 95% confidence interval: 0.63–0.87, $P < 0.0001$, F), were identified. In contrast, ET-1 was inversely correlated with SOD activity (r = -0.78, 95% confidence interval: -0.87 to -0.64, $P < 0.0001$, B).

of endogenous SOD (Table). Intracellular SOD has been shown to play a critical role in attenuating the intestinal inflammatory response.[26,17]

Propofol is widely used as an anesthetic before and during cardiac surgery, and as a sedative postoperatively in the intensive care unit. Also, in the clinical setting, pre-treatment with a certain drug for diseases related to intestinal I/R injury occurs with a higher frequency compared to other regimens, but sometimes treatment may be initiated after the onset of ischemia or during reperfusion due to unexpected occurrence of I/R event. In this regard, three different propofol regimens were used in the present study and a sedative dose of propofol was chosen to

investigate its effect on intestinal mucosal injury after intestinal I/R. Our results show that every propofol treatment regimen could significantly alleviate post-ischemic intestinal mucosal injury. However, propofol pre-treatment conferred the most profound protective effect. This is suggestive of a preconditioning-like effect of propofol, at least in the intestine. Ischemic preconditioning refers to a phenomenon in which a tissue is rendered resistant to the deleterious effects of prolonged ischemia by previous exposure to brief periods of vascular occlusion, and this preconditioning effect can be mimicked by pharmacological agents. Indeed, propofol has been shown to significantly increase heme oxygenase production in astrocytes and astroglial cells.[27,28] Heme oxygenase is a molecule with antioxidant properties that has been demonstrated to play a critical role in intestinal ischemic preconditioning that mediates protection against intestinal mucosal injury and the subsequent systemic inflammatory response.[29,30] Therefore, propofol may have initiated a preconditioning-like effect that is characterized by an increase of the endogenous antioxidant defenses, such as the increase of heme oxygenase and SOD activities. This is intriguing and merits further study.

It is known that oxidant stress is one of major factors contributing to intestinal I/R injury.[5,31] In our study, intestinal I/R injury was associated with a significant decrease of SOD activity, a major endogenous antioxidant enzyme, and increase of the lipid peroxidation product MDA in the injury group. Treatment with propofol increased SOD activity and attenuated MDA production that was associated with a reduced Chiu's score (Table). The significant positive correlation between tissue MDA content and Chiu's score (Figure 2A) is consistent with the notion that lipid peroxidation is a major cause of post-ischemic intestinal injury. A positive correlation between MDA and ET-1 (Figure 2C) as well as between MDA and NO suggests that the increase in lipid peroxidation is attributable, in part, to the increases of NO and ET-1. A recent study by Yagmurdur *et al.*[32] shows that propofol, but not the *iv* anesthetic ketamine, prevents burn injury induced increase in lipid peroxidation and attenuates gut mucosal epithelial apoptosis in rats, an effect that may be attributable to propofol antioxidant properties.

Although NO produced through constitutive NO synthase can be an important protective molecule for the small intestine at the onset of intestinal I/R,[15] over-production of NO through the inducible NO synthase (iNOS), especially under the circumstance of oxidant stress, may prove detrimental. The tight positive correlation between Chiu's score and NO production (Figure 2D) is consistent with the notion that over-production of NO could be detrimental. Under oxidant stress, the concurrent formation of high levels of superoxide and NO favour their reaction to form the potent oxidant peroxynitrite, resulting in further increased oxidative as well as nitrosative stress. Inhibition of iNOS has been shown to prevent the increase of NO production, reduce lipid peroxidation and attenuate intestinal I/R injury in the rats.[14,33] Propofol has been shown to suppress NO biosynthesis by inhibiting iNOS expression in lipopolysaccharide-activated macrophages[34] and inhibit the over-production of NO, leading to reduced vascular superoxide production and attenuated endothelial dysfunction in septic rats.[35] Further, propofol can react with peroxynitrite to form a propofol-derived phenoxyl radical, and therefore function as a peroxynitrite scavenger.[8] Although the effects of propofol on iNOS expression and phenoxyl radicals were not investigated in the present study, propofol inhibition of NO production (Table) suggests that its protective effect against intestinal I/R injury may be associated with the suppression of iNOS-NO-peroxynitrite pathway.

Endothelin-1 is an important participant in ischemia-reperfusion induced cardiovascular complications. Increased ET-1 activity is not only a causative factor to intestinal I/R injury,[17,36] but most importantly, elevated plasma ET-1 levels might be related to the size and extent of myocardial infarction and the mortality after myocardial infarction in patients,[37] a situation that is often accompanied with gastrointestinal complications.[38,39] Propofol attenuation of ET-1 production in the injured intestinal mucosa (Table) could potentially reduce its release to the circulation. It may also represent a mechanism whereby propofol attenuates MDA formation (Table), since ET-1 has been shown to stimulate superoxide production.[40] In addition, propofol reduction of the mucosa LD level, an index of anaerobic glucose metabolism, is indicative of improved intestinal mucosal microcirculation, which may be attributable to its effect in reducing ET-1, a potent vasoconstrictor.

In conclusion, we have shown that treatment, especially pre-treatment, with propofol at a sedative dose attenuates intestinal I/R-induced intestinal mucosa injury in an animal model. Further work is required to determine if this response translates to the bedside when propofol is used for conscious sedation following major cardiac surgery or for critical care patients at risk for gastrointestinal ischemia. The current results lend support to our previous hypothesis that propofol sedation might add to the beneficial effect of volatile anesthetic preconditioning.[41] Finally, in interpreting

these data, we caution that the intestinal ischemia-reperfusion insult that was examined in the current study is more severe than that which might be caused by CPB. In addition, although propofol is mainly absorbed into blood circulation after *ip* injection, it remains to be determined whether *ip* injection of propofol 50 $mg \cdot kg^{-1}$ could have produced a substantially larger concentration in the intestinal mucosa than would have resulted from a smaller dose of propofol administrated intravenously. Further studies in different animal models and in particular, in the clinical setting, are warranted.

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