

LABORATORY STUDY

Re-Protective Effects of Pre- and Post-Laparoscopy Conditioning, Zinc, Pentoxifylline, and N-acetylcysteine in an Animal Model of Laparoscopy-Induced Ischemia/Reperfusion Injury of the Kidney

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Background. Pneumoperitoneum (P) created to facilitate laparoscopy (L) is associated with splanchnic hypoperfusion, ischemia/reperfusion (I/R) injury, and oxidative stress. **Aim.** This study investigated the effects of pre- and post-laparoscopic conditioning, zinc, pentoxifylline (PTX), and N-acetylcysteine (NAC) on markers of I/R injury in an animal model. **Methods.** Sprague-Dawley male rats ($n = 56$, weight range 300–350 g) were randomly placed in one of seven treatment groups. Except for group C (control group who underwent a sham operation without pneumoperitoneum), pneumoperitoneum was created in all using CO₂ insufflation under a pressure of 15 mmHg. Group L (laparoscopy) was subjected to 60 min of pneumoperitoneum. Group Lpre (laparoscopic preconditioning plus laparoscopy) was subjected to 5 min of insufflation and 5 min of desufflation followed by 60 min of pneumoperitoneum. Group Lpost (laparoscopy plus laparoscopic post-conditioning) was subjected to 60 min of pneumoperitoneum and 60 min of desufflation followed by 5 min of insufflation and 5 min of desufflation. The laparoscopy plus zinc (LZ), PTX (LP), and NAC (LNAC) groups received a single intraperitoneal injection of zinc (50 mg/kg), pentoxifylline (50 mg/kg), or N-acetylcysteine (150 mg/kg) 5 min before the desufflation period. Animals were sacrificed at the end of the experiments, and kidney samples were tested for malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GPX), and superoxide dismutase (SOD). **Results.** MDA levels, as an indicator of oxidative stress in kidney tissue samples, were significantly higher in all pneumoperitoneum groups compared to Group C, except for Group Lpre. The pattern of change in tissue levels of SOD, GPX, and catalase was variable in the different treatment groups. **Conclusions.** In this animal model of renal ischemia/reperfusion injury, laparoscopy caused renal ischemia as evidenced by elevated markers of tissue ischemia-reperfusion injury. This effect was significantly attenuated by post-laparoscopy conditioning, zinc, pentoxifylline, and N-acetylcysteine, but not by pre-laparoscopy conditioning.

Keywords kidney, ischemia/reperfusion injury, oxidative stress, pneumoretroperitoneum, zinc

INTRODUCTION

Pneumoperitoneum facilitates the visualization of abdominal organs during laparoscopy. Despite the numerous advantages offered by this technique, experimental and clinical studies have shown that the increase in intra-abdominal pressure (IAP) accompanying laparoscopy-related pneumoperitoneum may cause oxidative damage of splanchnic and even remote organs.^[1] In animal studies, increases in oxidative stress markers were observed in blood plasma, intestine, liver, spleen, kidney, peritoneum, and even lungs following pneumoperitoneum with CO₂.^[1] In the clinical setting, laparoscopic procedures such as cholecystectomy^[2] and hernia repair^[3] induce oxidative

stress on tissues when compared with procedures performed through an open laparotomy incision.

Insufflation of gas into the peritoneum results in increased IAP with subsequent alterations in systemic, splanchnic, and renal circulation. These changes include a decrease in cardiac output and mean arterial pressure, transient elevations of aortic pressure and carotid arterial blood flow, and a reduction in blood flow in the portal vein, superior mesenteric artery, liver, spleen, pancreas, intestine, and kidneys.^[4] These circulatory changes are considered to be the main cause of oxidative damage to splanchnic organs.

Several strategies for the prevention of pneumoperitoneum-induced oxidative stress have yielded promising results: ischemic preconditioning,^[5] low intra-abdominal pressure,^[6] insufflation with helium,^[1] Trendelenburg positioning,^[7] pretreatment with erythropoietin,^[8] and the antioxidant melatonin.^[9] Effective prevention strategies are still needed because, to date, none of these approaches to prevent laparoscopy-related ischemic-reperfusion renal injury have been widely adopted. In this study, in an animal model, we tested the ability of pre- and post-laparoscopic conditioning, zinc, PTX, and NAC to prevent renal injury caused by pneumoperitoneum as measured by tissue markers of oxidative stress.

MATERIALS AND METHODS

The study protocol received approval from our university Animal Care and Ethics Committee. Sprague-Dawley male rats ($n = 56$, weight range 300–350 g) were housed in macrolon cages, three rats per cage, at 20–22°C under a 12 hour light:dark cycle, and were provided with a commercial pellet diet and tap water ad libitum.

The rats were randomly selected to one of seven treatment groups. Except for group C (the control group that underwent a sham operation without pneumoperitoneum), pneumoperitoneum was created in all using CO₂ insufflation as follows: rats were anesthetized with IM ketamine (50 mg/kg); then, after proper preparation of the surgical field, an 18 G Abbocath catheter was inserted into the abdominal cavity below the xiphoid process and connected to an insufflator tube.^[5] CO₂ was then instilled up to a pressure of 15 mmHg using an abdominal CO₂ insufflator (Karl Storz GmbH, Tutlingen, Germany). Group L (laparoscopy) was subjected to 60 min of pneumoperitoneum. Group Lpre (laparoscopic preconditioning plus laparoscopy) was subjected to 5 min of insufflation and 5 min of desufflation followed by 60 min of pneumoperitoneum. Group Lpost (laparoscopy plus laparoscopic postconditioning) was subjected to 60 min of pneumoperitoneum

and 60 min of desufflation followed by 5 min of insufflation and 5 min of desufflation. The laparoscopy plus zinc (LZ), PTX (LP), and NAC (LNAC) groups received a single intraperitoneal injection of zinc (50 mg/kg), pentoxifylline (50 mg/kg), or N-acetylcysteine (150 mg/kg) 5 min before the desufflation period.

Biochemical Analyses

Animals were sacrificed at the end of the experiments, and kidney samples were tested for malondialdehyde (MDA), acatalase (CAT), glutathione peroxidase (GPX), and superoxide dismutase (SOD) activities as follows: tissue specimens were washed with 0.9% NaCl to remove hematoma, dried, and stored in plastic bottles at 20°C until biochemical analyses were carried out. At the time of analysis, tissue samples were weighed, placed in 1.15% KCl solution, and homogenized for 15 minutes at 14,000 rpm. Aliquots of homogenates were centrifuged at 5,000 rpm for 30 minutes, and supernatants were analyzed for GPX, CAT, and SOD. MDA was measured by the modified Okawa method, absorbance of thiobarbituric acid-malondialdehyde.^[10] GPX was measured by nicotine amide adenine dinucleotide phosphate (NADP) reduction at 340 nm, and CAT was spectrophotometrically measured by the disappearance of H₂O₂ at 230 nm.^[11] SOD was determined as described by Fridovich.^[12] This method employs xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitro phenol-s-phenyl tetrazolium chloride) to form a red formazan dye. SOD is measured by the degree of inhibition of this reaction. The concentration of plasma lipid peroxidation (total MDA) was determined by the Okawa method with slight modifications. Protein was measured according to Lowry.^[13] Results were expressed in nanomoles per milligram protein (nmol/mg protein).

Statistical Analyses

All variables were expressed as medians and range. All data were entered into and processed by SPSS 9.05 for Windows® (SPSS, Inc., Chicago, Illinois, USA). Differences between groups were evaluated by Kruskal-Wallis variance analysis followed by post-hoc Mann-Whitney U testing. *p* values less than 0.05 were considered statistically significant.

RESULTS

Rats in all the groups survived until the end of the experiment. Levels of markers of oxidative stress taken

from the kidneys upon animal sacrifice are shown in Figures 1 through 4. MDA concentrations in group L were found to be significantly higher than those of groups C, LZ, LP, LNAC and Lpost (*p* < 0.05, see Figure 1). SOD concentrations in group L were found to be significantly lower than those of groups Lpre, LZ, LP, LNAC, and Lpost (*p* < 0.05, see Figure 2). GPX concentrations in group L were not significantly different than those of the other groups (*p* > 0.05, see Figure 3). The mean catalase concentration in group L was significantly lower than that of group C, but significantly higher than those of groups Lpre, LZ, and LP (*p* < 0.05, see Figure 4). Overall, levels of tissue markers of laparoscopy-related ischemia-reperfusion injury were higher in group L than in group C, and higher than in those groups that underwent laparoscopy but received protective pharmacological or pre- or post-conditioning treatment (see Figure 1). The pattern of change in tissue levels of SOD, GPX, and catalase was variable in the different treatment groups (see Figures 2–4).

DISCUSSION

Various tissue markers of oxidative stress have been measured to evaluate the effects of pneumoperitoneum on ischemia-reperfusion injury of splanchnic organs. Reactive oxygen species (ROS), overproduced during oxidative stress, are potent oxidizing and reducing agents that can directly damage cellular membranes by lipid peroxidation.^[14] Peroxidation of endogenous lipids leads to conversion of reduced glutathione to glutathione disulfide.^[15] MDA is an end product derived of peroxidation of polyunsaturated fatty acids and related esters. Thus, MDA tissue levels are a valid reflection of lipid peroxidation.

Another line of cellular defense against free radicals is a system of three enzymes, SOD, CAT, and GPX. SOD catalyzes the conversion of superoxides to hydrogen peroxide, which is subsequently converted to water and oxygen by CAT or GPX. Because it plays such a key role in cellular defense against free radicals, SOD is also an important indicator of the oxidative state.^[16] Although extensive research has demonstrated that ischemic preconditioning reduces renal ischemic-reperfusion injury, ischemic preconditioning is clinically feasible only when the occurrence of ischemia is predictable.^[17] Compared to the ischemic event, the onset of reperfusion is more predictable.^[17] Evidence of preconditioning as protection against ischemia-reperfusion injury in kidneys is still controversial, and of minimal benefit at best.^[18]

Ischemic postconditioning is a new, simple tool that can protect organs, including the kidneys, from I/R injury.^[19] Researchers have found that much of the post-ischemic injury occurs during the early moments of reperfusion, and

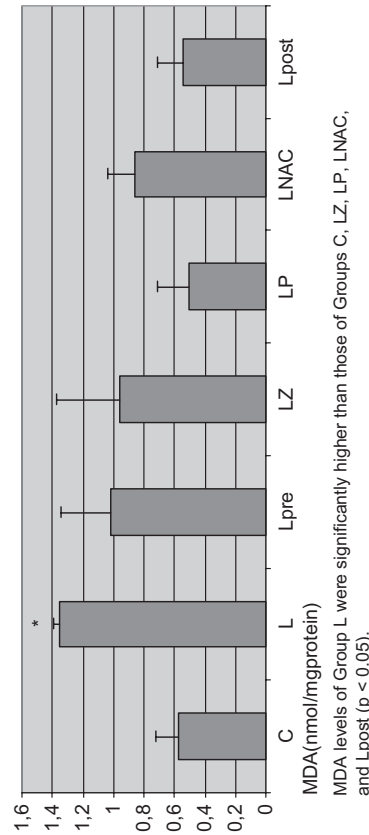


Figure 1. Malondialdehyde levels (nmol/mg protein) in kidney tissue after animals were sacrificed.

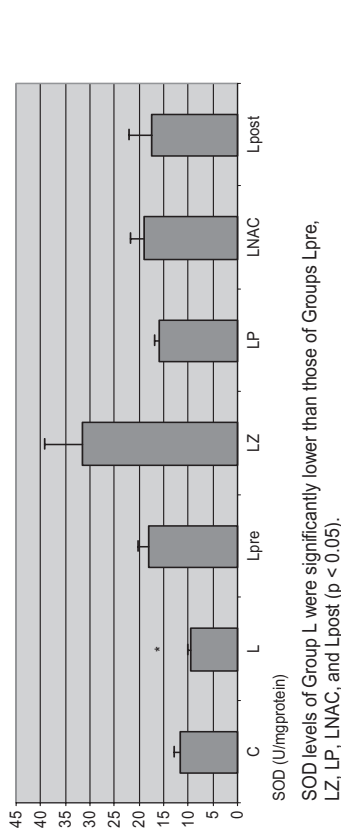


Figure 2. Superoxide dismutase levels (U/mg protein) in kidney tissue after animals were sacrificed.

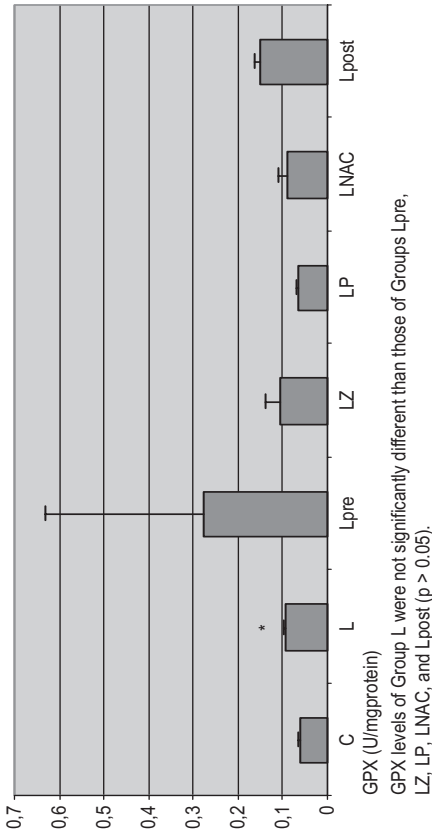


Figure 3. Glutathione peroxidase levels (U/mg protein) in kidney tissue after animals were sacrificed.

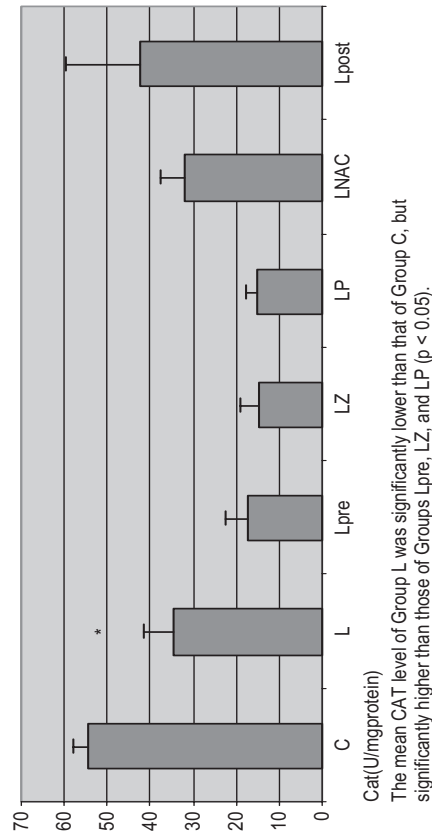


Figure 4. Catalase levels (U/mg protein) in kidney tissue after animals were sacrificed.

Figures 1–4. Levels of malondialdehyde (Figure 1), superoxide dismutase (Figure 2), glutathione peroxidase (Figure 3), and catalase (Figure 4) in kidney tissue after rats (n = 8 in each group) experienced sham without pneumoperitoneum (C), laparoscopy (L), laparoscopy with pre-laparoscopic ischemic conditioning (Lpre), IP zinc (50 mg/kg, LZ), IP pentoxifylline (50 mg/kg, LP), IP N-acetylcysteine (150 mg/kg, LNAC), and post-laparoscopic ischemic conditioning (Lpost).

that manipulation of this early reperfusion phase reduced I/R injury. Our results also supported these findings: ischemic post-conditioning attenuated renal injury after laparoscopy-induced I/R, as did pentoxifylline and N-acetylcysteine. Moreover, we demonstrated that ischemic postconditioning was better than ischemic preconditioning at reducing I/R injury of the kidney.

PTX, a methylxanthine derivative used for many years for its hemorheologically properties, has proven to be a potent inhibitor of tumour necrosis factor (TNF) production.^[20] Recent reports suggest that PTX can enhance the chemotactic response of neutrophils and may inhibit phagocytosis and superoxide production by neutrophils and monocytes.^[21] These laboratory findings have been translated into clinical benefits in attenuating I/R injury in patients with lung, intestinal, and kidney damage.^[22] To date, pentoxifylline has been shown to increase survival and preserve renal function after ischemia-reperfusion injury in a rat model. In our study, PTX lowered MDA and catalase levels and increased SOD in comparison to the laparoscopy group. Pentoxifylline lowered MDA levels more than the other methods.

NAC, a precursor of glutathione, has been used effectively to replenish intracellular glutathione stores in I/R conditions, and can directly scavenge radicals.^[23,24] In addition, it has a variety of antiinflammatory effects.^[23,24] Although many have investigated the preventive effect of NAC against renal I/R injury, its benefits in humans have yet to be proven. In our animal study, we found NAC to lower MDA and raise SOD levels compared with group L. In other words, NAC effectively attenuated the increase in MDA levels, which are caused by laparoscopy-related pneumoperitoneum.

Zinc has anti-inflammatory properties and, in cell culture and animals models, acts as an antioxidant. Its antioxidant properties arise from two mechanisms: it reduces redox-active metals, and it induces the antioxidant protein metallothionein, which can scavenge hydroxyl radicals.^[25] Other investigators claim that zinc exerts its tissue protective effects through other mechanisms, including DNA binding or inhibition of calcium influx.^[25] In our study, group LZ had significantly lower levels of MDA and SOD compared to the laparoscopy group, supporting its role in the reduction of I/R injury caused by laparoscopy-related pneumoperitoneum.

CONCLUSIONS

Laparoscopy with pneumoperitoneum at 15 mmHg causes increased oxidative stress in renal tissue. Zinc, pentoxifylline, N-acetylcysteine, and post-laparotomy conditioning significantly reduced markers of oxidative

stress caused by laparoscopy in this animal model. These findings should be tested in large-scale human studies with the aim of minimizing the morbidity and mortality of patients undergoing laparoscopy.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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