Comparison of the effects of low intra-abdominal pressure and ischaemic preconditioning on the generation of oxidative stress markers and inflammatory cytokines during laparoscopy in rats

Arif Serhan Cevrioglu1,5, Sezgin Yilmaz2, Tulay Koken3, Cigdem Tokyol4, Mehmet Yilmazer1 and Ibrahim Veyssel Fenkci1

Departments of 1Obstetrics and Gynaecology, 2General Surgery, 3Biochemistry, 4Pathology, Faculty of Medicine, Afyon Kocatepe University, 03200, Afyon, Turkey
5To whom correspondence should be addressed at: Department of Obstetrics and Gynaecology, Faculty of Medicine, Afyon Kocatepe University, 03200, Afyon, Turkey. Tel: +90 272 2136707 (ext.) 209; Fax: +90 272 2144996.

BACKGROUND: Pneumoperitoneum (Pp) induces ischaemia in intra-abdominal tissues. We investigated the effects of ischaemic preconditioning (IP) and low-pressure Pp methods used to reduce ischaemic injury during Pp on oxidative stress and inflammatory cytokine response. METHODS: Thirty-two rats were divided into four groups. Rats in the control group were subjected to only anaesthesia for 90 min. The other groups were subjected to Pp for 60 min with 15 (Pp15), 10 (Pp10) or 15 mmHg intra-abdominal pressure (IAP) after IP (IPPp15), all of which were followed by deflation (D) for 30 min. IP was defined as 10 min of Pp with 15 mmHg IAP followed immediately by 10 min of D. Peritoneum and blood samples collected at the end of the experiment were examined to determine inflammatory cytokine [tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)], oxidative stress [malondialdehyde (MDA)] and antioxidant [reduced glutathione (GSH) and glutathione reductase (GR)] levels. RESULTS: Blood and peritoneum MDA values and peritoneum TNF-α and IL-6 values decreased, while GR values increased in the Pp10 group in comparison with the Pp15 group. Blood and peritoneum MDA, TNF-α and IL-6 values decreased and GR values increased in the IPPp15 group in comparison with the Pp15 group. Blood MDA and IL-6 values in the IPP15 group were lower than those in the Pp10 group, whereas GR values were higher in the former. Except for high peritoneal IL-6 levels, no difference was found between the parameters in the IPP15 and those in the control group. CONCLUSIONS: IP may be more effective than low-pressure Pp in reducing ischaemic insult associated with laparoscopy.

Key words: cytokine/intra-abdominal pressure/ischaemia/laparoscopy/oxidative stress

Introduction

Improvements in video technology and surgical instrumentation have resulted in the current preference for minimally invasive techniques over traditional techniques for many surgical procedures. Despite its widespread use and increasing expertise in various kinds of surgical procedures, its adverse effects and haemodynamic consequences have not yet been fully elucidated.

It is reported that the intra-abdominal pressure (IAP) exerted for pneumoperitoneum (Pp) during laparoscopic operations, which is generally set at about 12–15 mmHg, can be increased to 20 mmHg in obese patients (Hulka and Reich, 1998). Several studies have demonstrated that IAP above the normal physiologic portal circulation pressure (7–10 mmHg) caused a marked reduction in blood flow to hepatic, renal and intestinal circulation (Chiu et al., 1995; Eleftheriadis and Kotzampasi, 1998; O’Malley and Cunningham, 2001; Richter et al., 2001; Neudecker et al., 2002). Although deflation restores visceral perfusion, it does not necessarily relieve oxidative stress in the tissue. Moreover, oxidative stress caused by reactive oxygen species (ROS) induced after the restoration of blood flow is one of the most important mechanisms contributing to organ dysfunction following ischaemia and reperfusion (I/R) injury (Li and Jackson, 2002). Therefore, organ dysfunction following Pp is caused not only by splanchnic or visceral ischaemia, but also by oxidative stress seen after I/R insult (Eleftheriadis et al., 1996; Seven et al., 1999; Glantzounis et al., 2001).

During laparoscopic operations the parietal peritoneum, as well as splanchnic organs, is exposed to ischaemic trauma. In humans an IAP of 15 mmHg decreased blood flow in the parietal peritoneum significantly, whereas no changes were
observed with pressures of 10 mmHg (Schilling et al., 1997). It was shown that carbon dioxide (CO₂), the gas most frequently used for Pp, led to local (intraperitoneal) and systemic acidosis and aggravated ischaemic organ injury further (O’Malley and Cunningham, 2001). The peritoneum, which includes a network of widespread capillaries and lymphs in the sub-mesothelial connective tissue, secretes many inflammatory mediators, vasoactive kinins, fibrins, and cytokines, and ROS such as superoxide anion, H₂O₂ and hydroxyl radical in response to I/R insults (Vittimberga et al., 1998; Holmdahl and Ivarsson, 1999; Gupta and Watson, 2001; Li and Jackson, 2002). In order to minimize the harmful effects that Pp with high IAP causes during laparoscopy it is recommended to select the minimal IAP value that will ensure sufficient visualization of the area to be operated on, rather than using the same Pp pressure value in all patients (Neudecker et al., 2002). Another method recommended for decreasing ischaemic injury associated with laparoscopy is ischaemic-preconditioning (IP), which is performed at the start of laparoscopy. IP is an endogenous protective mechanism by which short periods of I/R cycles may be followed by enhanced resistance to exacerbated cellular re-oxygenation injury (Peralta et al., 1999; Li and Jackson, 2002). Studies carried out on the assumption that an IAP increase and the following deflation during laparoscopy provided a model conducive to I/R showed that short periods of insufflation and deflation performed at the beginning of laparoscopy (mimicking the laparoscopic preconditioning) were effective in reducing ischaemic injury in intra-abdominal organs (Yilmaz et al., 2003a,b). The present study aimed to compare the effects of low-pressure Pp and IP methods on the reduction of I/R injury associated with pressure values normally used during laparoscopy.

Materials and methods

Study design
This study was an experimental, randomized, controlled trial with blind assessment of outcome.

Animals
Thirty-two female, non-pregnant Sprague–Dawley rats, weighing 264 ± 28 g, bred in isolation from male rats were included in this study. The animals were housed in the Animal Services Centre Laboratory at Afyon Kocatepe University. They were kept at temperatures between 20 and 25°C, with relative humidity between 40 and 70%, and 12 h light–dark cycles, and were given standard rat food and water ad libitum. The experiments were performed in accordance with the 1996 revised form of the guide for care and use of laboratory animals published by the United States National Institutes of Health. The study was approved by the University Ethics Committee. Unnecessary animal suffering was avoided throughout the study. At the beginning of the experiment, each rat was given a number, from 1 to 32. Then the rats were chosen randomly and put into separate cages for each group. The numbers of the rats in each cage were recorded by the principle author.

Anaesthesia
The rats were anaesthetized at the beginning of the experimental procedures by an intramuscular (i.m.) injection of ketamine (5 mg/kg, Ketalar®, Eczacibasi-Werner Lambert, Istanbul-Turkey). Rats that moved during the experiment were given an additional dose (1 mg/kg, i.m.) of ketamine.

Surgical technique
After stabilization of anaesthesia, the animals were placed in a supine position with the limbs secured to the table. The rats were randomly assigned to one of the four groups: Group Pp15 (n = 8) was subjected to 60 min of Pp with 15 mmHg of IAP followed by 30 min of deflation (D). Group Pp10, (n = 8) was subjected to 60 min of Pp with 10 mmHg IAP followed by 30 min of D. Group IPPp15 (n = 8) was subjected to the same Pp and D procedures as Group Pp15 after IP. For the purpose of this study, IP was defined as 10 min of Pp with 15 mmHg IAP followed immediately by 10 min of D. The control group (n = 8) was subjected to a sham operation at the end of the 90 min anaesthesia period, without Pp.

Group IPPp15 remained under anaesthesia for a total of 110 min (10 min Pp + 10 min D + 60 min Pp + 30 min D), whereas the total anaesthesia period in the other groups was 90 min.

Two rats in the Pp15 and IPPp15 groups moved during the experiment and were administered an additional dose of anaesthesia. During the Pp procedures one of the rats in the Pp15 mmHg group died. Another rat in the control group was excluded from evaluation upon the observation of a cancer-compatible mass of intestinal origin during laparotomy. As a result, the experiment was concluded with seven rats each in the Pp15 and control groups and eight rats each in the other two groups.

Creation of pneumoperitoneum
The abdomen was shaved with a safety razor and disinfected with polyvidone iodine solution (Batticon; Trommsdorff-Adeka Ilac Sanayi, Samsun, Turkey). A 1 cm midline incision was made beneath the umbilicus to allow access to the peritoneum. The system used to obtain Pp enabled simultaneous CO₂ insufflation into eight rats under the same pressure. This simultaneous insufflation procedure involved insufflating a cannula extending from the insufflator divided into right and left branches with the help of a stopcock. From each of these two branches, four auxiliary branches (a total of eight branches) were created, again by the use of stopcocks. The diameters of the main and auxiliary cannulae were equal. Thus it was ensured that carbon dioxide was given to all rats at the same time and pressure during insufflation (Akbulut et al., 2002;Yilmaz et al., 2003a, b). In order to make sure that there was no gas leakage from the cannulae, the ends of all cannulae were clamped before insufflation, the insufflator set at 15 mmHg was turned on and the pressure values were checked on the monitor. One end of a 25 cm long piece of plastic tubing was inserted into the peritoneal cavity as the vehicle for creating Pp, after which the incision was closed with a tight purse-string suture to prevent leakage of CO₂ from the abdomen. The other end of the tubing was connected to the CO₂ insufflator (Nortech, Model No: 3-315-00, Fribourg, Switzerland). The pressure of the CO₂ insufflator was fixed at 15 or 10 mmHg except for in the controls. An automatic insufflator provided CO₂ insufflation for the required intra-abdominal pressure. In the event of the intra-abdominal pressure decreasing due to transperitoneal CO₂ absorption or a possible gas leakage from the trocar entry site, the insufflator was automatically activated and pumped CO₂ into the abdominal cavities of the animals to keep the intra-abdominal pressure at the determined level.
**Tissue and blood sampling and preparation for further studies**

At the end of Pp and D in each group, the surgeons (the first and second authors) and their assistants divided the rats between the two teams and performed surgical dissection. After the Pp catheters were removed, laparotomy incisions were opened and extended, and 1 × 1 cm squares of parietal peritoneum examples from the right and left lower abdominal walls were grasped with atrumatic forceps and excised with scissors. Blood samples were taken from the intra-thoracic aorta with a 22 G needle attached to a 5 cc syringe. Blood samples were drawn into heparinized tubes. The rats were then killed with an intra-cardiac potassium injection while still under anaesthesia. It took 2 min on average to dissect the abdomen, collect blood and peritoneum samples and inject intra-cardiac potassium in each rat.

Half of the peritoneal tissue samples were washed with ice-cold lactated Ringer’s solution and stored at −20°C until analysis. The other half of the samples were immersed in 10% formaldehyde for later examination under the light microscope. Each tissue and blood sample was assigned a code number by the principal investigator and referred to the biochemists and pathologist participating in the study, who were blinded to the procedures applied to specific groups. All results were reported in relation to the sample code numbers.

**Biochemical analysis**

During the biochemical analysis, peritoneal tissue samples were initially homogenized (Ultra Turrax Homogenizer, T18 Basic, IKA, Wilmington, NC) in 0.1 M ice-cold phosphate buffer of pH 7.4. After homogenization, tissue samples were centrifuged at 3000 g and 4°C for 10 min. The supernatants were removed and used to determine oxidative stress markers and inflammatory cytokines. The tissue protein concentration was measured by the Biuret method. Results were expressed as µmol/g protein for malondialdehyde (MDA) and reduced glutathione (GSH), U/g protein for glutathione reductase (GR), and ng/g protein for tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6).

Blood samples were drawn into heparinized tubes during the experimental procedure. Plasma was separated by centrifugation at 800 g and 4°C over 10 min. Erythrocytes were washed three times with ice-cold physiological saline. The buffy coat together with part of the upper erythrocyte layer was removed and discarded after each washing step. After the washing procedure, the packed cells and plasma were stored at −20°C until analysis. The packed erythrocyte haemoglobin concentration was determined spectrophotometrically in lysed cells by the cyanomethaemoglobin method. Results were expressed as µmol/l for plasma MDA, and pg/ml for plasma TNF-α and IL-6.

Plasma and tissue MDA levels were determined by the thiobarbituric acid method of Ohkawa et al. (1979). MDA is an indicator of oxidative stress, since it results from the breakdown of lipid peroxyl radicals. MDA is also important as it can cause further oxidative injury by oxidizing protein molecules (Stadtman and Berlett, 1997). Despite these advantages, MDA reflects changes in numerous other biochemical systems as well as ROS, in particular the prostaglandins. Therefore, in the evaluation of oxidative stress we examined not only MDA, but also markers showing antioxidant status (GR and GSH). GR enzyme plays a part in the further reduction of the GSH, which is bound to the free oxygen radical (ROS), inactivates it and becomes oxidized in the process (Meister, 1994; Li and Jackson, 2002). Erythrocyte and tissue reduced GSH levels and GR enzyme activities were determined as an indicator of erythrocyte or tissue antioxidant capacity, using the methods described by Beutler et al. (1957) and Goldberg and Spooner (1983). Erythrocyte and tissue GR activities were measured with a Hitachi 917 autoanalyser using commercial kits obtained from Randox Laboratories (Randox Laboratories Ltd, County Antrim, UK). Plasma and tissue cytokine (TNF-α and IL-6) levels were determined as an indicator of the activation of the systemic immune system, using a commercially available rat ELISA kit (Biosource Europe SA, Nivelles, Belgium). Other chemicals used in the evaluation of tissue and blood GSH and MDA were purchased from the Sigma Chemical Co. (St Louis, MO).

**Histopathological examination**

Tissue samples were fixed in 10% neutral buffered formalin. Samples were embedded in paraffin, cut into sections 3 µm thick and stained with haematoxylin–eosin. One section of each peritoneal tissue sample was systematically analysed by a pathologist blinded to the study groups. Each section was evaluated for intra-cellular oedema, congestion, haemorrhage and interstitial inflammatory cell infiltration, using the semiquantitative scale described by Hauet et al. (1997). Sample results were reported under their respective code numbers. The total sum of histopathological scores was obtained for all groups by combining the individual parameters (Table I).

**Statistical analysis**

Data were analysed with the Statistical Package for the Social Sciences, version 10.0 (SPSS Inc., Chicago, IL). Values are expressed as mean ± standard error of the mean. A non-parametric statistical test was used. Differences between the two study groups were tested by Mann–Whitney U test. A P-value of < 0.05 was considered statistically significant.

**Results**

The results for oxidative stress markers and inflammatory cytokines are displayed in Figures 1 to 5.

**Plasma and tissue MDA levels**

Comparisons among the groups revealed that the highest plasma MDA values were in the Pp15 group, followed by the Pp10, IPPp15 and control (C) groups, in descending order (P < 0.001; Pp15 vs Pp10 and IPPp15, and C). Plasma MDA values in the Pp10 group were higher than those in the IPPp15 (P < 0.05) and control (P < 0.001) groups. No difference was found between plasma MDA values of the IPPp15 and control groups.

**Table I. Details of the histopathological grading system used to evaluate peritoneal tissue samples**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>No abnormality</td>
</tr>
<tr>
<td>2</td>
<td>Mild lesions affecting 10–25% of the HPFE</td>
</tr>
<tr>
<td>3</td>
<td>Lesions affecting 25–50% of the HPFE</td>
</tr>
<tr>
<td>4</td>
<td>Lesions affecting 50–75% of the HPFE</td>
</tr>
<tr>
<td>5</td>
<td>Lesions affecting more than 75% of HPFE</td>
</tr>
</tbody>
</table>

HPFE: High power field examined. Peritoneal tissue samples were examined for intracellular oedema, congestion, interstitial haemorrhage and nonspecific inflammatory cell infiltration according to the injury grading scale (Hauet et al., 1997).
C. Peritoneal MDA, (b) Pp10, and IPPp15, and C; (c) Pp groups, whereas they were not statistically different from Pp with 10 mmHg IAP (Group Pp10), or Pp with 15 mmHg IAP after ischaemia preconditioning (Group IPPp15). Mean scores ± SEM are indicated. Plasma MDA, (a) $P < 0.001$ vs Pp10, and IPPp15, and C; (c) $P < 0.05$ vs IPPp15; (d) $P < 0.001$ vs C. Peritoneal MDA, (b) $P < 0.001$ vs Pp10, and IPPp15, and C.

The highest peritoneal tissue MDA values were in the Pp15 group, followed by the Pp10, IPPp15 and control groups, in descending order ($P < 0.001$; Pp15 vs Pp10, and IPPp15, and C). There was no apparent difference among the Pp10, IPPp15 and control groups in terms of peritoneal MDA values (Figure 1).

**Erythrocyte and tissue GR levels**

When the groups were examined in terms of erythrocyte GR activities, values in the Pp15 group were lower than those in the IPPp15 and control groups ($P < 0.001$), but there was no statistically significant difference between the values in the Pp10 and Pp15 groups. Erythrocyte GR values in the Pp10 group were lower than those in the IPPp15 ($P < 0.05$) and control ($P < 0.001$) groups. No difference was found between erythrocyte GR values in the IPPp15 and control groups.

Peritoneal GR values in the Pp15 group were lower than those in the Pp10 ($P < 0.05$), IPPp15 and control groups ($P < 0.001$). There was no statistically significant difference between peritoneal GR values in the Pp10 and IPPp15 groups, but the values of the former were significantly lower than those in the control group ($P < 0.01$). No difference was found between peritoneal GR values in the IPPp15 and control groups (Figure 2).

**Erythrocyte and tissue GSH levels**

An examination of the groups in terms of GSH showed that the highest level of consumption was in the Pp15 group followed by the Pp10, IPPp15 and control groups, in descending order. Despite the numerical difference, there was no statistical difference among the groups with regard to erythrocyte GSH levels. However, the peritoneal tissue samples revealed that consumption in the Pp15 group was significantly higher than that in the control group only ($P < 0.01$) (Figure 3).

**Plasma and tissue TNF-α levels**

Plasma TNF-α values in the Pp15 group were higher than those in the IPPp15 ($P < 0.05$) and control ($P < 0.001$) groups, whereas they were not statistically different from those in the Pp10 group. Plasma TNF-α levels were significantly higher in the Pp10 group than those in the control ($P < 0.01$) group, whereas they were not statistically different from those in the IPPp15 group.

Peritoneal TNF-α values were higher in the Pp15 group than those in all other groups ($P < 0.05$), but no difference was determined between peritoneal TNF-α values in the Pp10 group and those in the IPPp15 and control groups. No difference was found between peritoneal TNF-α values in the IPPp15 and control groups (Figure 4).

**Plasma and tissue IL-6 levels**

A comparison of the groups in terms of IL-6 values showed that plasma values in the Pp15 group were higher than those in the IPPp15 and control groups ($P < 0.001$), whereas they were not statistically different from those in the Pp10 group. Plasma IL-6 levels in the Pp10 group were significantly higher than those in the IPPp15 ($P < 0.05$) and control ($P < 0.01$) groups. There was no difference in plasma IL-6 values between the IPPp15 and control groups.
Peritoneal IL-6 values were significantly higher in the Pp15 group than those in the Pp10 ($P < 0.05$), IPPp15 and control groups ($P < 0.001$). Peritoneal IL-6 levels in the Pp10 group were higher than those in the control ($P < 0.01$) group only. Peritoneal IL-6 values in the IPPp15 group were higher than those in the control group ($P < 0.05$) (Figure 5).

**Histopathological findings**

Light microscopy revealed no evidence of overt injury in any peritoneal tissue sample. Despite a small numerical difference, there was no statistical difference among the groups in terms of the histopathological scoring system (Table II).

**Discussion**

An imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to functional organ deterioration, is termed ‘oxidative stress’ (Sies, 1997). Overall, responses to surgery in general are reflected in terms of immune function (Vittimberga et al., 1998; Gupta and Watson, 2001) and oxidative stress (Eleftheriadis et al., 1996; Li and Jackson, 2002). The appearance of a certain amount of inflammatory cytokine and ROS in the tissue after surgical trauma and the organism’s elimination of these substances over time are considered a physiological reaction. However, in the event of excessive production of such substances and the failure of natural scavengers to eliminate them, a process starts that can lead to cellular apoptosis and necrosis (Sies, 1997). Therefore, obtaining less inflammatory cytokine and a smaller oxidative stress response in the post-operative period is deemed beneficial to the patient.

Values of the markers that were studied in both blood (plasma and erythrocyte) and parietal peritoneum samples that indicated oxidative stress (MDA) and cytokine response (TNF-α and IL-6) were higher in the group receiving 15 mmHg IAP. In addition, levels of the markers showing anti-oxidant activity (erythrocyte GSH and GR) were lower in this group. It was seen that IP, a manoeuvre used to reduce surgical trauma, provided an evident decrease in oxidative stress and inflammatory cytokine response alike, while low-pressure (10 mmHg) Pp was not as effective as IP (Figures 1 to 5).

Lower-pressure Pp procedures were tried in animal and human models to reduce tissue ischaemia associated with high-pressure Pp. In a laparoscopic cholecystectomy using a gasless technique and 14 and 10 mmHg pressure, Giraudo et al. (2001) compared hepatic function test results and showed that transaminase values increased more in the group operated on with 14 mmHg IAP, while transaminase values in the 10 mmHg and gasless groups were similar. Samel et al. (2002) demonstrated in a rat model that intra-abdominal pressures of 10 and 15 mmHg caused a significant stepwise decrease in the jejunal mucosa perfusion index (29 and 78%, respectively), resulting in a severe impairment of mucosal microcirculation. However, there are also studies in rat (Yilmaz et al., 2004) and human (Polat et al., 2003) models showing that no statistically significant difference could be
found in oxidative stress response between 10 and 15 mmHg pressure applications.

In the present study, a comparison of the Pp15 and Pp10 groups in terms of oxidative stress and inflammatory cytokine response demonstrated that there was a significant difference ($P < 0.001$) between the groups only in terms of MDA, which was studied in both plasma and peritoneum, and that there was either no difference in terms of GSH levels or the difference was only in peritoneum samples in terms of GR, TNF-α and IL-6 parameters ($P < 0.05$). Our findings suggest that low-pressure Pp can be effective to a degree in reducing oxidative stress and inflammatory cytokine response, particularly at the peritoneal level.

The second manoeuvre we employed to reduce ischaemic injury associated with laparoscopy was IP. This is an injury-limiting mechanism initially described as the attenuation of cardiac damage due to a severe I/R insult by previous short I/R cycles (Murry et al., 1986). The liver and kidney have also been shown to benefit from IP (Peralta et al., 1999; Ogawa et al., 2000). However, in these experiments a mechanical obstruction was performed on the vascular pedicle of the studied organ to induce ischaemic insult, whereas we evaluated laparoscopic Pp applied at 15 mmHg pressure as the ischaemic insult model (Yilmaz et al., 2003a,b). The most important piece of information that guided authors of the study in this procedure was that when IAP values used for Pp were above normal portal venous pressure (7–10 mmHg), this would lead to ischaemia in intra-abdominal organs by reducing portal blood flow and organ circulation would return to normal after deflation (Richter et al., 2001; Samel et al., 2002). Thus, it was speculated that the use of short-term Pp and deflation before long-term Pp could create an IP effect on splanchnic organs, and especially the parietal peritoneum.

Experiments conducted to demonstrate the efficacy of IP in reducing long-term ischaemia and the following reperfusion (I/R) injury have various IP application times, but IP was generally performed in the form of 5–10 min of ischaemia, followed by 5–10 min of reperfusion (Sola et al., 2000; Clavien et al., 2000; Gonj et al., 2004). Reinheckel et al. (2000) showed in a rat model that protein carbonyls, a marker of oxidative stress, are detected in mitochondrial proteins after 10 min hypoxia and 5 min re-oxygenation. Based on the exemplary IP times used in the literature, the ischaemia (insufflation) and reperfusion (deflation) times of the IP procedure in this experiment were determined to be 10 min each. It was seen that the levels of the markers that were studied in both blood and peritoneal tissue and that showed oxidative stress and inflammatory response were similar in laparoscopic IP and control groups, but the levels in the former were significantly different in comparison to those in the Pp15 and Pp10 groups (Figures 1, 2, 4 and 5). Our findings support the thesis that the IP procedure carried out in the experiment made the rats more resistant to the next and longer ischaemic stimulation.

Raising intra-abdominal pressure leads to ischaemia and the formation of ROS in the peritoneum in a time- and pressure-level-dependent manner (Diebel et al., 1992; Li and Jackson, 2002). When aerobic conditions are restored after reperfusion, ROS increase and scavenger antioxidant substances decrease initially, but tissue antioxidant defences build up over time and eliminate high levels of ROS from the medium (Li and Jackson, 2002). Some cellular proteins released during ischaemic preconditioning are also known to make the cell resistant to the harmful effects of ROS. For instance, preconditioning causes over-expression of the anti-apoptotic gene product called heat shock 70 (HSP70) in coronary endothelial cells and this reduces hypoxic injury (Suzuki et al., 1998). Our finding suggests that substances released from the peritoneum in response to short-term ischaemia in the group to which preconditioning was applied made tissues more resistant to the next and longer ischaemia, and thus made oxidative stress and inflammatory cytokine response milder.

Although the pressure we applied (10 mmHg) to determine the effect of low-pressure Pp is below 15 mmHg, it still has the potential to cause ischaemia since it is above the 7–10 mmHg normal intra-abdominal pressure value (Richter et al., 2001). It was shown that even if an IAP value (5 mmHg) lower than the normal IAP value interval were used, it could cause oxidative stress (Bentes de Souza et al., 2003b). This finding implies that oxidative stress response might stem from the gas used (CO2) rather than IAP during Pp. It was reported that hypercapnia and acidosis that developed as a result of trans-peritoneal absorption of the CO2 used in Pp could bring about vasoconstriction and increase vascular resistance and hypo-perfusion (O’Malley and Cunningham, 2001). Yesildaglar and Koninckx (2000) showed in a rabbit endoscopic surgery model that increasing IAP values used during CO2 Pp (5 vs 20 mmHg), as well as increasing insufflation flow rates (1 l/min vs 10 l/min), enhanced mesothelial hypoxaemia and therefore peritoneal adhesion formation. However, the haemodynamic effects caused by CO2 Pp on human tissues may be different from those on rat tissues. Bentes de Souza et al. (2003a) compared peritoneal MDA levels in gynaecological operations performed with laparoscopy and open surgery and showed that while there was no marked MDA increase in the laparoscopy group, there was an increase in MDA in the laparotomy group. This finding suggests that peritoneal tissue response levels of humans to laparoscopic ischaemia even at similar IAP values may be different from those of rats.

It may be beneficial to use intubation and mechanical ventilation as well as blood gas follow-up and close haemodynamic monitoring, especially in experiments in which a high intra-abdominal pressure model is used, in order to reduce evaluation errors that could result from differences in the gas used in Pp, the insufflation system, and the intra-abdominal volume of the subject. Mynbaev et al. (2002) showed in a rabbit endoscopic surgery model that CO2 Pp with 10 mmHg IAP profoundly affected blood gases and acid base homeostasis, resulting in metabolic hypoxaemia. Since we did not perform intubation and mechanical ventilation and did not follow up blood gases during the experiment, we cannot tell whether hypercapnia or elevated intra-abdominal pressure influenced the rise in MDA and inflammatory cytokines. Nonetheless, despite the fact that the IPPp15 group...
and Pp15 group were subjected to the same IAP values during the Pp, oxidative stress and inflammatory cytokine response in the IPPp15 group were similar to those in the control group, showing that IP can be beneficial even in such circumstances.

The fact that we collected blood and tissue samples only at the end of the experiment in order to show oxidative stress and inflammatory cytokine response prevented us from following the synthesis and elimination cycles of these markers at different stages of the experiment. More objective data could have been obtained if blood and peritoneum levels of these markers had been examined at the beginning of the experiment, during Pp and after deflation. Since we conducted our study on rats, we did not have the opportunity to place trocars in the abdomen during the experiment or to collect peritoneal tissue samples at various times. In order to reduce the risk of misinterpretation that could result from our failure to identify at one single time the reactions associated with an ischaemic insult, we used multiple oxidative stress and inflammatory response markers. Similarly, we evaluated both blood and peritoneum samples at the same time and thus we were able to study the correlation between local and systemic responses. Furthermore, the fact that the parameters we studied were at similar levels in the blood and peritoneum showed that the parietal peritoneum could provide valuable information reflecting the general response of the organism.

Another aspect of our findings that could be criticized is that although there were differences among the groups in terms of oxidative stress and inflammation markers studied in tissue homogenates, there were no signs of ischaemic injury in the peritoneal tissue examinations carried out with the light microscope (Table II). Our literature search did not reveal another study presenting light microscopy findings of ischaemic injury caused by Pp in the peritoneum compared with oxidative stress and inflammatory responses in the peritoneal tissue homogenates. It is possible that the changes we found in oxidative stress and inflammation markers in peritoneal tissue homogenates were accompanied by tissue damage that could only be revealed with electron microscopy in its early stages.

In conclusion, the findings of our experiment suggest that ischaemia preconditioning applied in the form of 10 min pneumoperitoneum and deflation periods can be more effective than low-pressure pneumoperitoneum in reducing the oxidative stress and inflammatory cytokine response associated with laparoscopy. Our experimental protocol of preconditioning may be too lengthy to be recommended for direct use in humans. Nonetheless, studies can be conducted to identify the minimum period that will suffice to reveal the effect of preconditioning.

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