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CO_2 Pneumoperitoneum Prolongs Survival in an Animal Model of Peritonitis Compared to Laparotomy

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Background. **The advantages of laparoscopic surgery have been well documented. However, the impact of pneumoperitoneum on sepsis sequelae is still equivocal. This study aimed to evaluate the effect of CO_2 pneumoperitoneum, applied under different pressures and exposure times, on sepsis cascade and mortality.**

Material and methods. **In 42 New Zealand rabbits, peritonitis was induced by the cecum ligation and puncture model. After 12 h, the animals were randomized in seven groups: a control group, four groups with pneumoperitoneum (10–15 mmHg for 60–180 min), and two groups with laparotomy (for 60 and 180 min). Blood samples were collected before cecum ligation and puncture, 12 h later and 1, 3, and 6 h after pneumoperitoneum desufflation or abdominal trauma closure to evaluate bacteremia, endotoxemia, white blood cells count, C-reactive protein, and procalcitonin levels. Furthermore, the mortality time was recorded in all animals.**

Results. **Bacteremia and endotoxemia were induced in all groups. Endotoxemia levels were significantly more elevated in the group where pneumoperitoneum was performed under 15 mmHg for 180 min compared with all other groups at 1 and 3 h after pneumoperitoneum desufflation ($P < 0.05$), except when compared with the group where pneumoperitoneum was performed under 10 mmHg for 180 min. White blood cell and C-reactive protein levels showed similar trends for all groups. However, serum procalcitonin reached statistically higher levels ($P < 0.05$) in groups with laparotomy compared with groups with pneumoperitoneum and with the control group at 6 h. Survival was lower in the laparotomy groups compared with the pneumoperitoneum groups and with the control group ($P < 0.05$).**

Conclusions. **In the presence of peritonitis, CO_2 pneumoperitoneum applied in clinically standard pressures, even for extended time intervals, reduces the severity of sepsis and prolongs survival.**

Key Words: **pneumoperitoneum; laparoscopy; endotoxemia; C-reactive protein; procalcitonin; sepsis; peritonitis; mortality; survival.**

INTRODUCTION

It has been well documented that surgical trauma impairs the immune functions. Specifically, major operations are associated with an initial intense hyperinflammatory response followed by a transient immune suppression which, under certain circumstances, can progressively lead to sepsis, multiple organ dysfunction, or even death [1, 2]. The majority of previous experimental and clinical studies have shown a distinct superiority of laparoscopic over open surgery regarding the preservation of immune functions [3, 4]. This superiority is demonstrated by several observations, including less postoperative pain and faster recovery. Nevertheless, it is still unclear whether the immunological superiority of laparoscopic techniques over conventional procedures is maintained under septic conditions. This issue is critical, considering that there has been an increase in the application of lapa-

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roscopic surgery in cases characterized by localized or diffuse peritonitis, such as acute appendicitis, perforated peptic ulcer, and diverticulitis.

In 2005, the European Association of Endoscopic Surgery presented, in a consensus statement, the guidelines for the role of laparoscopic surgery in abdominal emergencies, based on an extended review of the existing literature [5]. According to these guidelines, laparoscopic surgery should be preferable over open surgery to treat patients with a presumable diagnosis of perforated peptic ulcer, acute cholecystitis, appendicitis, and pelvic inflammatory disease, although its role in other cases of intra-abdominal infection, such as acute diverticulitis, is not clear yet. However, two recent studies, a review article from the Cochrane database [6] and a meta-analysis from the French Association of Digestive Surgery [7], have concluded that laparoscopic appendicectomy significantly increases the risk of postoperative intra-abdominal abscess formation. In addition, there has been disagreement between studies regarding the negative effect of pneumoperitoneum in peritonitis, with some of them demonstrating an increased risk for bacterial translocation and bacteremia when pneumoperitoneum is established in a septic environment [8], and others showing the superiority of laparoscopic surgery in preserving the local and systemic immune response even under septic conditions [9].

Due to the ambiguity and inconclusiveness of the previous findings, this study was conducted to investigate the impact of pneumoperitoneum, induced in various intra-abdominal pressures and for different time intervals, on bacteremia, endotoxemia, systemic inflammatory response, and survival in a peritonitis model.

MATERIALS AND METHODS

All experiments were performed in the Laboratory of Surgical Research of the 2nd Surgical Department of the Aristotle University of Thessaloniki. The study was approved by the National Animal Ethical Committee and followed the national laws on the care and use of laboratory animals, which are in conformance to the directive of the European Union.

Animals

Forty-two white male New Zealand rabbits, age 3.4 ± 0.4 mo and weight 2.8 ± 0.2 kg, were used. The animals were housed in individual cages where standard chow and water were available *ad libitum*. The rabbits were acclimatized to their environment for 5 days after their arrival and then fasted for 12 h before the beginning of the procedures. The animal housing environment was kept at a temperature of 22°C with a 12-h light/dark cycle.

Experiments

All surgical procedures were conducted under aseptic conditions. The introduction to anesthesia was performed with intramuscular injection of ketamine (Ketaset, 35 mg/kg) and xylazine (Xylapan, 5 mg/kg), while the maintenance of anesthesia was achieved with intramuscularly administered xylazine when necessary. The animals were allowed to breathe spontaneously during the experiment. The study included two phases, A and B.

Phase A

Before the beginning of Phase A, a blood sample of 6 mL was drawn through a puncture of the right ear vein of each animal. Each rabbit was shaved with a clipper and the abdomen was painted with a 10% provide iodine solution. A 6-cm incision was made in the midline and the peritoneal cavity was entered through the linea alba.

In all animals, peritonitis was established using a previously described model of peritonitis-cecal ligation and puncture (CLP) in rats [10], which was modified in this case for rabbits. CLP consisted of a dissection of the cecum, a ligation midway between the ileocecal valve and the terminal cecum using a 0-0 silk tie, and a triple puncture of the isolated cecum with a 14-gauge needle to cause a lethal sepsis [11]. The cecum was then returned to the abdominal cavity and the abdominal wall was closed in one layer with a running absorbable suture of polydioxane no. 2/0 (Prolene, Ethicon Inc., Sommerville, NJ), while the skin was closed with a running suture of polypropylene 2-0 (Prolene, Ethicon Inc.). At the end of Phase A, the animals were transferred to their cages where they received fluid resuscitation with repeated (ever 4 h) subcutaneous injections of lactated Ringer's solution (10 mL/kg).

Phase B

Phase B of the experimental protocol started 12 h after the end of Phase A. During this phase, the animals were randomized in seven groups as shown below:

- Group 1 (n = 6); animals underwent no further intervention (control group).
- Group 2 (n = 6); pneumoperitoneum under 10 mmHg for 60 min was established.
- Group 3 (n = 6); pneumoperitoneum under 10 mmHg for 180 min was established.
- Group 4 (n = 6); pneumoperitoneum under 15 mmHg for 60 min was established.
- Group 5 (n = 6); pneumoperitoneum under 15 mmHg for 180 min was established.
- Group 6 (n = 6); re-laparotomy was performed through the previous abdominal trauma, with exposure of the abdomen to ambient air for 60 min, followed by closure of the abdominal wall in one layer with a running absorbable suture of polydioxane no. 2/0 (Prolene, Ethicon Inc.) and by closure of the skin with a running suture of polypropylene 2-0 (Prolene, Ethicon Inc.).
- Group 7 (n = 6); re-laparotomy was performed through the previous abdominal trauma, with exposure of the abdomen to ambient air for 180 min, followed by closure of the abdominal trauma in one layer with a running absorbable suture of polydioxane no. 2/0 (Prolene, Ethicon Inc.) and by closure of the skin with a running suture of polypropylene 2-0 (Prolene, Ethicon Inc.).

In groups 2 to 5, pneumoperitoneum was achieved by introducing a Veress needle into the peritoneal cavity and by insufflating the abdomen with CO_2 using the Wisap CO_2-Pneu Semm System gas insufflator (Wisap, Sauerlach, Germany). In all groups, except for the control group, blood samples of 6 mL were collected from the right ear vein at the following time points:

- Immediately before peritoneum establishment in groups 2 to 5, and immediately before re-laparotomy in groups 6 and 7;
- 1 h after pneumoperitoneum desufflation in groups 2 to 5, and 1 h after closure of the abdominal trauma in groups 6 and 7;
- 3 h after pneumoperitoneum desufflation in groups 2 to 5, and 3 h after closure of the abdominal trauma in groups 6 and 7;
- 6 h after pneumoperitoneum desufflation in groups 2 to 5, and 6 h after closure of the abdominal trauma in groups 6 and 7.

In the control group 1, blood samples were obtained at the begin-