Innovative technique for the study of pneumoperitoneum in the evaluation of abdominal pain after chemical irritation with zymozan

Técnica original para o estudo da influência do pneumoperitônio na avaliação da intensidade da dor abdominal após irritação química com zimozan

Eduardo CremaI, Bruna Ferrante SilvaII, Pauliana Lamounier e SilvaIII, Virmondres Rodrigues JúniorIV, Fernando CunhaV

I PhD, Full Professor, Department of Digestive Surgery, Federal University of Triângulo Mineiro (UFTM), Uberaba - MG, Brazil.
II MD, Postgraduate Program of Surgery, UFTM, Uberaba - MG, Brazil.
III Scientific Initiation Students, UFTM, Uberaba - MG, Brazil.
IV Full Professor, Immunology, UFTM, Uberaba - MG, Brazil.
V Full Professor, Pharmacology, UFTM, Uberaba - MG, Brazil.

ABSTRACT

Purpose: To assess the influence of pneumoperitoneum in mice submitted to peritoneal irritation provoked by the biological agent Saccharomyces cerevisiae, by counting the number of abdominal contractions elicited.

Methods: To study the effects of pneumoperitoneum analgesic action, 60 mice were divided into two groups: the experimental group, subjected to pneumoperitoneum; and the control group, without pneumoperitoneum. The both groups received intraperitoneal injection of zymosan at a dose of 1mg/0,2ml/mouse.

Results: The sum of the number of abdominal contractions of the experimental group (with pneumoperitoneum) was significantly lower than that of the control group (without pneumoperitoneum). In the experimental group, a lower number of contractions occurred in each min compared to the control.

Conclusion: The observation of the analgesic effect of pneumoperitoneum using CO2 in mice submitted to peritoneal irritation by zymosan was verified.

Key words: Laparoscopy. Zymosan. Pneumoperitoneum, Artificial. Abdominal Pain. Mice.

RESUMO

Objetivo: Avaliar a influência do pneumoperitônio em animais submetidos à irritação peritoneal provocada pelo agente biológico Saccharomyces cerevisiae, mediante a contagem do número de contrações abdominais.

Métodos: Para o estudo do efeito da ação analgésica do pneumoperitônio os 60 camundongos foram divididos em dois grupos, grupo experimento (com pneumoperitônio) e controle (sem pneumoperitônio). Os dois grupos receberam injeção intraperitoneal de zimossan na dose de 1mg/0,2ml/camundongo. Resultados: O somatório do número de contrações abdominais do grupo experimento (com pneumoperitônio) foi significativamente menor que no grupo controle (sem pneumoperitônio). O número médio de contrações no grupo controle foi significativamente maior quando comparado com o grupo experimento. Conclusão: Observou-se efeito analgésico do pneumoperitônio com CO2 em animais submetidos à irritação peritoneal pelo zimossan.


Introduction

Currently, laparoscopic procedures have gained importance due to their advantages in relation to open surgery. Among these advantages, diminished postoperative pain, a reduction in respiratory complications, reduced hospital admittance and an early return to normal activities can be cited1,2,3.

To perform laparoscopic surgery, separation of the abdominal wall from the abdominal viscera is required and the most commonly used technique is pneumoperitoneum, which is realized with CO2 insufflation in the intraperitoneal cavity1,2,4.

When laparoscopy was first performed, pneumoperitoneum was treated as prejudicial, since it diminished venous return, causing CO2 retention, later, studies demonstrated that pneumoperitoneum maybe responsible for the enormous benefits of laparoscopic surgery by liberating inflammatory cytokines (IL-4 and IL-10), which are potent analgesics1,5,6.

Among patients that require analgesia, low-pressure pneumoperitoneum reduces the demand for anesthetic agents. The analgesic effect of pneumoperitoneum is maintained for 3-5 postoperative days, further contributing to better quality of life in these patients7.

The beneficial effects of insufflation have been related to mastocyte degranulation, responsible for the liberation of vasoactive substances (histamine, adrenalin) and cytokines (IL-4 and IL-10), denominated as antiinflammatory, which have been shown to limit the inflammatory response to pain, inhibiting the production of hyperallergic cytokines and prostaglandins8.
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Even so, no consensus exists regarding the benefits of its use, since it induces adverse reactions in cardiovascular and pulmonary function, despite the fact the hemodynamic repercussions have been reduced with the use of low-pressure pneumoperitoneum and are not statistically significant.

To study inflammatory and pain reaction, the use of peritoneal irritants, such as thioglycollate, sephadex and zymosan, have been used by several authors.

This work aimed to assess the influence of pneumoperitoneum in mice submitted to peritoneal irritation provoked by the biological agent *Saccharomyces cerevisiae*, by counting the number of abdominal contractions elicited.

**Methods**

Seventy male BALB/c mice were selected, aged 30-35 days and weighing between 20-25g, reared in the animal center of the Triangulo Mineiro Federal University. All the surgical procedures followed the criteria, technical norms and international laws regarding research on animals determined by the Ethics Committee of the Brazilian College of Animal Experimentation (Comitê de Ética do Colégio Brasileiro de Experimentação Animal, COBEA), with the approval of the Ethics Advisory Committee of the same institute (Brazilian Association for Animal Studies; Associação Brasileira para Estudos Animais).

To study the effects of pneumoperitoneum analgesic action, 60 mice were divided into two groups: the experimental group, subjected to pneumoperitoneum; and the control group, without pneumoperitoneum.

All the procedures were realized under inhalatory anesthetic, using diethyl ether.

The technique used to perform the pneumoperitoneum included puncture of the median line of the anterior abdomen wall with a no. 18 (18-G) angiocath. Insufflation with CO2 was maintained by a digital insufflator (Karl Storz Co., Culver City, CA, USA), at a constant pressure of 5 mmHg for 20 min. A system was developed with a disposable 3-via tap and two latex extensions, permitting the maintenance of pneumoperitoneum in four mice simultaneously. The mice were anesthetized by nasal cone lined with cotton-wool soaked in ether. After 20 min, insufflation was interrupted and the injected CO2 was removed from the abdominal cavity by catheter with auxiliary digital compression and a change in mouse body position.

Aimed at assessing the analgesic effect of pneumoperitoneum, chemical irritation of abdominal cavity was realized by the biological agent *Saccharomyces cerevisiae* (zymosan), at a dose of 1 mg/0.2ml/mouse, prepared immediately prior to the experiment. The introduction of zymosan was realized along the median line of the anterior abdomen wall with a 1 ml syringe attached to a needle.

The experimental group (n=30) was divided in two subgroups of 15 mice each, which received intraperitoneal injection of zymosan 1 and 2 hours, respectively, after the end of pneumoperitoneum. The control group was also divided into two subgroups of 15 mice each, which received peritoneal irritation by the same technique and dose 1 and 2 hours after only being anesthetized.

After the zymosan injection, one mouse from the experimental group and one from the control group were placed in individual glass bell jars. For a period of 10 min, the number of contractions was observed min by min, which were standardized as the extension of the four paws and a clear persistent contraction of the abdomen. The contractions were quantified by an individual counter for each mouse. For each min, the number of contractions was registered in an individual file and totaled after 10 min.

Pain evaluation was quantified by the number of abdominal contractions obtained for each group, such that these numbers were compared inter and intragroup for each min and later submitted to statistical analysis.

**Statistical analysis**

The variable of interest, number of abdominal contractions, was initially submitted to the Kolmogorov-Smirnov normality test followed by Bartlett’s test of homogeneity of variances. Intergroup comparisons for each min of observation were realized by the Mann-Whitney test and, intragroup comparisons between min were realized by Friedmann’s test, followed by the Tukey test. The total number of contractions in the first 10 min was also considered, with comparative analysis realized by the Student t test.

**Results**

All the mice submitted to the experiment presented good evolution.

As shown in Figure 1, the sum of the number of abdominal contractions of the experimental group (1 and 2h, with pneumoperitoneum) was significantly lower (11.47) than that of the control group (22.93; 1 and 2h, without pneumoperitoneum).
Intragroup comparative analysis of the number of contractions after 1 and 2 h demonstrated that no significant difference occurred when comparing the experimental or control groups (Figure 2).

Discussion

The majority of laparoscopic procedures performed involve pneumoperitoneum. When laparoscopy was first performed, the maintenance of positive intraabdominal pressure throughout the laparoscopic procedure was considered nonphysiological and associated with adverse metabolic, immunological, hemodynamic and oncological effects3,4.

Several clinical papers have shown the benefits of laparoscopic surgery in relation to the open surgery option2,6, most likely resulting from the liberation of cytokines IL-4, IL-10 and IL-131,2.

As has been shown in large randomized studies, low-pressure pneumoperitoneum (7 mmHg) leads to diminished pain and a 20% reduction in the number of patients requiring analgesia in the short-term postoperative period, when compared to individuals submitted to standard pneumoperitoneum pressure of 12 mmHg10,11.

Given that pneumoperitoneum is a crucial element of laparoscopy, guidelines were established in order to reduce its adverse effects, such as a preoperative evaluation of the presence of cardiac, pulmonary, hepatic, renal and vascular comorbidities. Continuous monitorization of pressure levels so that they remain as low as possible, sufficient to achieve good exposure of the surgical field. The use of 2-5mm trocars, rather than 5-10mm, was also established, contributing to diminished postoperative pain.

Certain peritoneal irritants have been used to study pain mechanisms, such as acetic acid and zymosan, which, minutes after injection into the abdominal cavity, provoke motor manifestations of abdominal contraction and limb extension; manifestations that can be easily quantified by a researcher and translate the abdominal pain felt by experimental animals12.

In an attempt to assess the real analgesic effect of pneumoperitoneum in animal experimentation, this study used a product that provokes peritoneal irritation (zymosan), as proven in cytokine/pain study protocols3,13,14.

Observation revealed that mice that were submitted to pneumoperitoneum presented a significantly lower mean number (11.47) of abdominal contractions (pain) than control group mice (22.93) after peritoneal irritation with zymosan (Figure 1).

Upon analyzing the moments 1 and 2 h after the realization of pneumoperitoneum prior to peritoneal irritation by zymosan, observation also showed that the mean number of contractions was statistically lower compared to the control group (without pneumoperitoneum). However, intragroup comparison of the pneumoperitoneum group at 1 and 2 h revealed no statistically significant difference in contractions (Figure 2), demonstrating the lasting effect of the analgesic action of pneumoperitoneum, probably resulting from the liberation of anti-inflammatory substances (cytokines IL-4 and IL-10), as previously observed in a study on patients submitted to laparoscopic cholecystectomy1.

In all the mice studied, observation showed that peritoneal irritation was immediate, given that the onset of abdominal contractions occurred within the first min, increased up to 3 min, after which a tendency of decreased contractions occurred...
up to the end of 10 min studied; thus proving that the method is simple, cheap and easy to execute in the study of abdominal pain using peritoneal irritants. Similar observations were verified in a study by Vale et al.15.

At all times, from the first min onward, the analgesic effect of pneumoperitoneum was observed in virtue of the significantly lower mean number of contractions in the experimental group subjected to pneumoperitoneum.

Conclusion
Observation of the analgesic effect of pneumoperitoneum using CO2 in mice submitted to peritoneal irritation by zymosan was verified.

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Correspondence:
Eduardo Crema
R. Marcos Lombardi, 305
38050-170 Uberaba – MG Brazil
Phone: (55 34)3318-5288 / 9972-3883
cremaufm@mednet.com.br

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