Original Article

Influence of pneumoperitoneum on tumor implantation in parietal incision: a comparative study of trocar incision and laparotomy in rats

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ABSTRACT: Videolaparoscopic surgery has enabled great progress in surgical procedures. However, the literature shows great controversy concerning its indication in oncological surgery for possibly increasing the implantation of metastases. This experimental study was carried out to help solve this controversy and analyze the influence of pneumoperitoneum as a cause of this implantation. A comparative study between incisions of trocars of videolaparoscopy and conventional laparotomy was conducted. For this analysis, 30 Wistar rats were divided into two equal groups: laparotomic group (A), that underwent a 5 cm laparotomy, which was left exposed for 15 minutes; and another (B), in which a 3 mmHg pneumoperitoneum was created with CO_2 and two more trocars were inserted, maintaining the insufflation for the same period. In both groups, the tumor, Walker 256 carcinosarcoma, was inoculated in the surgical procedure. The evaluation was performed within the fifth and the seventh postoperative days. The result of the macroscopic evaluation indicated that the tumor invasion in group A reached 93.33% and the implantation in the laparoscopy incision reached 73.33%. Nevertheless, the histopathological exam showed tumor implantation in all incisions of both groups (100%). The study concluded that parietal tumor implantation is not influenced by pneumoperitoneum in incisions of trocars when compared with conventional laparotomy in rats.

Keywords: laparoscopy; laparotomy; neoplasm metastasis; implantation; carcinosarcoma.

RESUMO: A cirurgia videolaparoscópica proporcionou grande avanço nas técnicas cirúrgicas. No entanto, há controvérsia na literatura sobre sua indicação na cirurgia oncológica por possivelmente aumentar a implantação tumoral de metástases. No intuito de contribuir para esclarecer essa controvérsia, realizou-se este estudo experimental visando analisar a influência do pneumoperitônio como causa dessa implantação. Realizou-se um estudo comparativo entre incisões dos trocartes da videolaparoscopia e da laparotomia convencional. Para isso, foram utilizados 30 ratos Wistar divididos em dois grupos iguais: um laparotômico (A), que foi submetido à laparotomia com 5 cm de extensão, a qual ficou exposta por 15 minutos; e outro (B), no qual se efetuou pneumoperitônio de 3 mmHg com CO_2 e colocaram-se mais dois trocartes, mantendo-se insuflado pelo mesmo período. Em ambos os grupos, inoculou-se o tumor no ato operatório, o carcinossarcoma 256 de Walker. A avaliação deu-se entre o quinto e sétimo dia de pós-operatório. Como resultado na avaliação macroscópica, há invasão tumoral no grupo A de 93,33% e implantação na incisão laparoscópica de 73,33%. No exame histopatológico, porém, revelou-se implantação tumoral em todas as incisões de ambos os grupos (100%). Conclui-se que a implantação tumoral não sofre influência do pneumoperitônio nas incisões dos trocartes comparadas com a laparotomia convencional em ratos.

Palavras-chave: laparoscopia; laparotomia; metástase neoplásica; implantação; carcinossarcoma.

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INTRODUCTION

In early 20th century, Kelling conceived an endoscopic method, adapting a cystoscope to examine the peritoneal cavity[1].

With the progress and development of special cameras, Mouret, a French gynecologist, performed the first videolaparoscopic cholecystectomy in 1987. In Brazil, De Paula and Szego performed the first videolaparoscopic procedures in 1989[1].

The increasingly more frequent use of this procedure is due to better postoperative recovery of patients, such as: lower doses of analgesics; shorter period of adynamic ileum, which enables early oral nutrition; easy mobility and early deambulation; shorter hospitalization period; quick return to socioeconomic activities and better esthetic result[2-14].

Several studies have demonstrated that laparoscopy can be used in oncologic surgeries, but only long-term results after using this surgical technique can actually clarify this controversy and establish its full acceptance in the treatment of gastrointestinal cancer[3,4,6-8,13-57].

Local, locoregional, parietal or diffuse recurrences have been similar in both surgical techniques. However, data are not yet conclusive, due to short follow-up period and reduced number of patients analyzed[4,11,33,41,43,49,54,58].

The literature shows great controversy regarding parietal tumor implantation, which would be greater at sites of surgical trocar introduction, due to the presence of pneumoperitoneum, when compared to rates of implantation from incisions in conventional laparotomy, with CO_2 possibly causing some impact on the immunological function[19,23,24,31,37-39,42,43,50,58-81].

Studies have shown that free tumor cells in the abdominal cavity also contaminate incised and healed tissues that had no contact with instruments or tumors; then, its hematogenic dissemination is improbable[33,43,52,57,59,82].

Carcinosarcoma

Walker 256 carcinosarcoma was discovered in 1928 (Earle, 1935) from a breast adenocarcinoma in a female rat. It is one of the few types of tumor available for experimental studies, with the advantage of being easily obtained *in vivo* and presenting quick development, but with the disadvantage of presenting great aggressiveness, very quick development and short latency period[58,83-93].

Objectives

Analyze the influence of pneumoperitoneum with CO_2 on parietal tumor implantation, conducting a comparative study between incisions of videolaparoscopy trocars and incision of conventional laparotomy in rats.

MATERIAL AND METHODS

Material – experiment animal

The study used 30 male Wistar rats of 180 to 200 grams, from the Central Biotery at the Universidade Federal de São Paulo. The animals were transported in proper vehicle and cages and remained at the Biotery of Surgical Technique and Experimental Surgery at the Pontifícia Universidade Católica in Campinas (accredited by the Colégio Brasileiro de Experimentação Animal).

Methods

Material obtained for tumor inoculation

Three rats were obtained from the biotery at the Universidade de São Paulo, which were inoculated with Walker tumor and, eight days later to allow tumor development, ascitic fluid with tumor cells was removed and then inoculated in the studied rats.

Access approach

All 30 rats adapted to biotery were randomly divided into two identical groups, named A and B, with 15 rats each. The animals were anesthetized with ethyl ether, submitted to trichotomy and abdominal antisepsis with PVPI.

All 15 rats of group A were submitted to median laparotomy of 5 cm length, inoculating the ascitic fluid with viable tumor cell, around 1 mL with $3x10^5$, directly in the abdominal cavity, which remained exposed for 15 minutes. After that, the incision was sutured with complete stitches, separated, keeping 5 mm spacing, with non-absorbable 4-zero suture.

All 15 rats of group B were submitted to puncture in the left hypochondrium using a Veress needle and then to pneumoperitoneum, through intra-abdominal insufflation of carbon dioxide, until reaching intra-

cavitary pressure of 3 mmHg. After such pressure was reached, two 3-mm trocars were introduced using new punctures, one in the right flank and on in the left flank of the abdomen, with inoculation right after the tumor, with the same characteristics of the other group, using one of the trocars. Pneumoperitoneum and two intra-abdominal trocars were kept for 15 minutes. After this period, pneumoperitoneum was emptied through the trocars, which were removed and then the incisions were sutured with one complete stitch of non-absorbable 4-zero suture.

The postoperative progress of both groups of rats was monitored four times a day, for max. seven days, submitting the rats that had not died until then to new anesthesia for abdominal evaluation and then to euthanasia.

The evaluation of parietal tumor implantation in the rats analyzed the abdominal wall resection to check for tumor implantation in surgical incisions in both groups, ending with the removal of abdominal organs with suspicion of any macroscopic alteration.

The rats that died before the seventh day were analyzed through the same method as that applied to rats that lived until the end of the experiment.

RESULTS

Table 1 shows the abdominal cavity evaluation in terms of macroscopic invasion of parietal incision.

Tables 2 and 3 show other macroscopic findings in the rats of groups A and B, respectively.

The material was collected from areas with probable tumor implantation and incisions of both groups were resected, even without macroscopic evidence of implantation. All material was submitted to histopathological analysis.

Neoplasm was developed in all examined samples, except in the retroperitoneum biopsy of rat #26.

The histopathological analysis showed, in all samples, neoplasm of solid mass of intermediate sized cells, with clear cytoplasm and basophils, large nuclei, with grumous chromatin and prominent nucleoli.

Such neoplasm infiltrated the subcutaneous region, adipose and muscular tissue, delaminating the muscular fibers.

Practically all samples had focus of necrosis; in some cases, it was extensive, without viable neoplastic cells, like the samples from incisions of rats #5, 7, 10, 20, 21, 24, 26 and 27.

The material collected from the peritoneum of rats #5, 28 and 30 and from the retroperitoneum of rats #22, 27 and 30 presented focus of necrosis.

The samples from incisions of rats #23 and 25, and from the retroperitoneum of rat #23 presented extensive necrosis, but they also had viable neoplastic areas.

At first, this experimental study would include the statistical analysis of results, but it became unnecessary, as neoplasm developed in all samples from parietal incisions in both groups.

DISCUSSION

After the initial excitement with the advent of laparoscopic surgery and its various advantages, such as reduced postoperative pain and recovery period, as well as early discharge from hospital and return to daily activities, it presented significantly reduced utilization in oncological colorectal surgery, as demonstrated by the frequency of recurrent lesions and presence of tumor at trocar insertion sites. However, recent studies report infrequent occurrence of these complications, and not only in laparoscopy, as they have been reported in open surgery too.

No consensus is seen also in the pathogenesis of metastatic implantations, as it involves multiple factors. Tumor cells exfoliated during the surgery may be the origin of cell implantation at the surgical wound site in both laparoscopy and open surgery. Studies have demonstrated that tumor cells can spread through the peritoneum before and after intestinal tumor resection. The incidence of positive results for tumor cells in the peritoneum ranges between 0 and 42%, depending on the time in relation to the surgery (pre- or postoperative period), tumor stage and detection capability of the technique used for tumor cells[94].

Clinical studies have shown the possibility of videolaparoscopy techniques presenting higher incidence of parietal metastasizing. Pneumoperitoneum, for contributing to a phenomenon called aerosolization, is the most questioned factor for this fact[39,66,70,73,74,78,81,94]. However, the relation between pneumoperitoneum and the forms of tumor cell dissemination and tumor implantation is not clear[4,11,33,41,43,45,49,54,68].

In an attempt to eliminate this controversy, an experimental study was conducted with rats to evaluate the influence of pneumoperitoneum in tumor implantation in parietal incision, as this is one of the main factors that distinguish laparoscopy from laparotomy, and as it plays an important role in tumor cell dissemination in parietal incision of trocars.

For this reason, Walker 256 carcinosarcoma was used. The literature indicates $1x10^7$ and $2x10^5$ viable tumor cells as adequate for intraperitoneal inoculation in Wistar rats, as this amount produces ascites and death within approximately seven days[58,86,87,92].

The fact of tumor dissemination observed in all animals of the experiment can be explained by the formation of new blood vessels[95-97], as it occurs in normal healing process of surgical wounds, and by the loss of cohesion between tumor tissue cells[98] and increased cell motility[99], resulting from tumor manipulation in rats of group A of laparotomy, and by the flow of gas in pneumoperitoneum in group B.

Although the incidence of tumor implantation in surgical incision is real, after the laparoscopic resection of a carcinoma, it is not definitively establis hed[4,11,33,41,43,45,49,54,68], estimated to be within the range of 0 to 21%[67,71,76]. However, recent studies report values of 0.6 to 0.7 of incisional tumor recurrence after laparotomy[43,52,57,59]. In addition, with the time, the range of incidence has decreased, also suggesting that the surgeons' experience influences on the occurrence of such implantations.

Three prospective randomized studies examined the recurrence of lesions, comparing laparoscopy and open surgery. Stage et al. monitored 18 patients for 14-month follow-up and found no lesion recurrence or implantation at the access ways; Lacy et al. reported the same results when monitoring, for 21 months, 91 segmental resections, 44 of them made via laparoscopy; and Milson et al. reported two cases of recurrent lesion in the abdominal wall associated with generalized disease. These are small samples, and, therefore, with the idea of limited result, but they demonstrate acceptable oncological results[100].

Regarding the presence of carcinomatosis and tumor implantation in surgical incision, Le Moine et al. conducted a compared study of abdominal tumor growth in a surgical wound, in experiment animals submitted to tumor resection through laparoscopy or laparotomy. Despite similar indexes of metastases in surgical wound, the number of peritoneal carcinomatosis in the laparoscopy group was higher. Then, the authors concluded that pneumoperitoneum had harmful effects in terms of tumor dissemination in the peritoneal cavity. However, all rats of that experiment were submitted to exploratory laparotomy with incision of 5 cm length, before they were randomized. Three trocars were introduced in the rats of pneumoperitoneum group soon after the incision closure. Then, the rats were submitted to 12 mmHg pressure or pneumoperitoneum with CO_2 for 20 minutes, followed by tumor resection. This way, the rats in the pneumoperitoneum group were submitted to laparotomy associated with high pressure of insufflation, and this additional stress may have affected the final result of the group and have mistakenly altered the study conclusions. In contrast, in this study, tumors were inoculated in the pneumoperitoneum group through one of the trocars, not requiring the use of laparotomy. However, the study of Le Moine et al. and this study presented similar results when the surgical wound of both groups were compared[77].

Innumerous clinical studies report the implantation of malignant disease in trocar wound after laparoscopic surgery for the treatment of ovarian cancer and hepatocellular, gastric, pancreatic, biliary vesicle and colon carcinomas[4,10,15,19,23,31,33,37,38,63-65,79,100]. Some authors also believe that recurrences would be a result of the advanced disease, and peritoneal carcinomatosis, in response to this hypothesis, would be related to the laparoscopic technique. However, some articles describe such implantations in resections of non-advanced type T1 tumor and even tumors not diagnosed in initial laparoscopic procedures[4,10,23,31-33,37,50,63-65,69,76,79,100].

Other authors believe that tumor implantation are associated with the process of tumor extraction using trocars[19,23,31,37,63,67,77,100]. However, the literature reports tumor implantation even in trocar wounds not used to extract surgical specimens[77,82].

Another assumption would be that the CO_2 used in abdominal insufflation or physical effects of pneumoperitoneum would cause reduced immunological function, thus, allowing tumor implantation in trocar wounds[39,66,70,73-75,78,81,94]. However, implantations in abdominal wall, in laparotomy, have random and

rare occurrences. Studies show that patients died due to early dissemination of the disease, making authors consider recurrences in abdominal wall as the result of disseminated disease and not an isolated tumor implantation[94,101]. In this study, such assumption was not confirmed, with similar results in both groups.

Tumor staging has a direct influence on the postoperative result of tumor implantation incidence and recurrences, and it should be taken into account as a variant when comparing patients submitted to different techniques. A retrospective study with 5-year follow-up analyzed 110 patients submitted to laparoscopic colectomy due to colorectal cancer to determine long-term results. It conclude that survival for each stage is not different in relation to open surgery, stage I (94% vs. 72% to 74%), stage II (66% vs. 54% to 63%), stage III (55% vs. 39% to 46%) and stage IV (0% vs. 6% to 7%). According to the author, implantations in wound may be overestimated in laparoscopy or underestimated in open surgery, recurrences can also be part of a disseminated disease. In this series, no incisional implantations were observed at trocar sites. Just as in other series, 1% was reported, or less, which is very close to the implantation rate in wounds in open surgery – 0.6% to 1.5%[102].

Moreira JR et al. (2000) conducted an experiment: they placed filters in laparoscopy trocars, with analysis of different insufflation pressure measures, performing gas exhaust and subsequent count of tumor cell concentration in the filters placed in various trocars, compared to laparoscopy, which used parietal retraction. They demonstrated the possibility of tumor cell aerosolization, indicating that it may occur during the laparoscopic procedures, even without pneumoperitoneum; thus, suggesting other mechanisms involved. Abdominal lifting, either through retraction or CO_2 insufflation, may cause cell aerosolization, regardless of the pneumoperitoneum pressure. They also suggested that increased pneumoperitoneum pressure may not cause aerosolization, but it would be associated with increased contamination by surgical instruments and recurrence of incisional tumors. In addition, increased contamination of these instruments and access ways would probably be the result of a pressure-dependent mechanism, which does not include aerosolization. This mechanism was not exactly described, and the study also reported that events causing tumor recurrence may involve multiple factors: increased pressure may not only result in greater number of tumor cells, involving also incised tissues, but also molecular and/or physiological alterations to the incision site, ensuring selective advantages of growth and survival to cells[94-96,101-106]. But, as demonstrated in our study, no difference was observed in both surgical accesses in tumor implantation in parietal incisions of the groups, showing that aerosolization, i.e., tumor dissemination and subsequent implantation, is not directly related to pneumoperitoneum.

Although several techniques have been used to demonstrate aerosolization, no floating tumor cell has been demonstrated through the analysis of CO_2[107].

It is believed that CO_2 promotes tumor cell growth, but in vitro studies have analyzed alterations caused by CO_2 in tumor growth and have demonstrated that it does not actually occur. The results suggest that CO_2 of at least 10 to 15 mmHg will cause a toxic effect[107].

Regarding the fact that videolaparoscopy may replace the conventional laparotomy access in the treatment of intra-abdominal tumors, the question remains very complex. But, with the results of this study, we believe that it is necessary to change the "laparotomy x laparoscopy" focus and consider these as specific techniques, following scientific criteria of indication, considering the equipment availability and skills for the proper execution of each approach aiming at the rational application of both techniques.

Through a review on current literature and sources such as SCIELO and LILACS, we can conclude that the use of videolaparoscopy as a proper oncological technique remains controversial, especially because most studies in this area are retrospective and only suggest conclusions, without conditions to be adopted as a support to practices, and prospective randomized studies are required to monitor patients for long postoperative periods, attesting the occurrence or non-occurrence of higher incidence of recurrence at trocar incision sites and lesion recurrence[107-111].

CONCLUSION

The results of this study indicate that pneumoperitoneum did not influence tumor implantation in trocar incisions, when compared to laparotomy.

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