Effects of Carbon Dioxide Pneumoperitoneum, Air Pneumoperitoneum, and Gasless Laparoscopy on Body Weight and Tumor Growth

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Background: The oncologic consequences of intraperitoneal carbon dioxide (CO2) insufflation during the laparoscopic resection of cancer are under debate. The effect of other insufflating gases or gasless laparoscopy on cancer requires study.

Objective: To study body weight and tumor growth in rats after CO2 pneumoperitoneum, air pneumoperitoneum, and gasless laparoscopy.

Methods: On day 1, an 8-mg bolus of ROS-1 tumor was placed under the renal capsule of both kidneys in rats. In experiment A, rats had either CO2 insufflation (n=10) or a gasless laparoscopic bowel resection (n=10) on day 3 and were humanely killed after 7 days. In experiment B, rats had either a laparoscopic bowel resection with CO2 insufflation (n=11) or insufflation with air (n=11) on day 3 and were killed after 7 days. In both experiments, postoperative weight loss and tumor growth were measured, and the differences were tested with an analysis of covariance.

Results: Renal subcapsular tumor growth in the group having gasless laparoscopy was less than that in the group having CO2 pneumoperitoneum (P=.04). Postoperative weight loss in these groups showed no differences (P=.55). No differences in tumor growth or weight loss were found between rats having insufflation with CO2 and those having insufflation with air (P=.61 and P=.68, respectively).

Conclusions: The restoration of body weight after a laparoscopic surgical procedure was similar with CO2, air, or gasless laparoscopy. Gasless laparoscopy was associated with less renal subcapsular tumor growth than was insufflation with CO2. Therefore, the application of gasless techniques in laparoscopic oncologic surgical treatment demands further study.

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Laparoscopic techniques are progressively used in surgical practice for both benign and malignant disease. Although minimally invasive surgical treatment has become the preferential approach to gallstone disease, hiatal esophageal disorders, and small adrenal tumors, the value of minimally invasive techniques in the treatment of malignant disease remains unresolved.1-4 Major concern has been caused by more than 20 published reports of tumor recurrences at the site of cannula insertion or at the site of the extraction of the specimen after laparoscopic resection for cancer.5,6 Experimental studies have shown, however, that tumor retrieval and growth are less after a laparoscopic surgical resection than after an open surgical procedure.7,8 This apparent oncologic benefit of laparoscopic surgical resection has been attributed to reduced surgical trauma that is associated with this approach. One of the drawbacks of laparoscopic surgical therapy is that the intraperitoneal insufflation of carbon dioxide (CO2) is required to create a working space. Insufflating CO2 into the peritoneal cavity results in hypercarbia, acidosis, hemodynamic alterations, and gut ischemia.9,10 The metabolic and oncologic consequences of CO2 pneumoperitoneum have not been studied extensively until now. Alternatives to CO2 pneumoperitoneum are the use of other insufflation gases or mechanical elevation of the abdominal wall (gasless laparoscopy). The effects of CO2 and increased abdominal pressure on cancer are unknown and demand further study.

The aim of this study is to assess body weight and tumor growth after the intraperitoneal insufflation of CO2 compared with air or gasless laparoscopy.
MATERIALS AND METHODS

ANIMALS

Thirty-four male inbred WAG-Rij-strain rats (Harlan-CPB, Austerlitz, the Netherlands) weighing 260 to 330 g aged 3 to 4 months were used. The rats were bred under specific pathogen-free conditions. They were housed in freestanding cages and acclimated to standard laboratory conditions (temperature, 20°-24°C; relative humidity, 50%-60%; 12 hours of light and 12 hours of dark). The rats were fed a standard laboratory diet (Hope Farms, Woerden, the Netherlands) with unlimited access to water and food before and after the surgical procedure. The experimental protocols adhered to the rules in the Dutch Animal Experimental Act of 1977 and the Guidelines on the Protection of Experimental Animals published by the Council of the European Community (1986). The protocol was approved by the Committee on Animal Research of the Erasmus University, Rotterdam, the Netherlands.

TUMOR

The ROS-1 osteosarcoma (transplantable to WAG-Rij rats) was used. This osteosarcoma originated spontaneously in the tibia of a rat. The tumor was maintained in vitro in RPMI-1640 medium supplemented with 5% fetal calf serum (screened for virus and Mycoplasma species), 1% penicillin (5000 U/mL), 1% streptomycin sulfate (5000 U/mL), and 1% levoglutamide (200 mmol/L) (Gibco, Paisley, England). Before their use, cells were treated with trypsin (5 minutes at 37°C), centrifuged (5 minutes at 3000 revolutions per minute), resuspended in RPMI-1640 medium, and counted. Viability was measured with trypan blue exclusion (0.3% in a solution of 0.9% sodium chloride). Viability always exceeded 95%. To grow solid tumor, 2 × 10⁶ ROS-1 tumor cells were injected in the right and left flanks of syngeneic WAG-Rij rats. After 3 weeks, the tumor in both flanks reached a volume of 2.5 cm³, and the tumor mass was aseptically separated with a scalpel from the outer membrane of the main lesion. The tumor was then cut into 1-mm³ cubes of about 8 mg, immersed in a culture solution, and stored at 4°C until the solid bolus was placed under the renal capsule. All cubes were placed underneath the renal capsule within 1 to 4 hours after specimens of the solid ROS-1 tumor were obtained from syngeneic WAG-Rij rats.

OPERATIVE PROCEDURES

After the rats were anesthetized with atropine sulfate (Centrafarm, Etten-Leur, the Netherlands), 0.05 mg/kg of body weight; a sedative, 0.25 mg/kg intramuscularly (Domitor, SmithKline Beecham Pharmaceuticals, Zoetermeer, the Netherlands); and 100-mg/mL ketamine hydrochloride, 40 mg/kg intraperitoneally (Ketalin, Apharmo, Arnhem, the Netherlands), the abdomen of the animals was shaved. The rat was secured to the operating table in a supine position with adhesive tape, and the abdomen was cleaned with 70% alcohol and dried with gauze.

On day 1, all rats underwent a 2.5-cm midline laparotomy. The kidneys were exposed, and 8 mg of solid ROS-1 tumor was placed under the capsule of each kidney under microscopic vision. During the operation, the viscera was covered with phosphate-buffered saline-wetted gauze. The operative time of this procedure varied between 20 and 25 minutes. The viscera was returned to the abdominal cavity, and the abdomen was closed in 1 layer using a running suture.

On day 3, all rats were weighed and underwent a laparoscopic procedure. The laparoscope, camera, and attached cables were held at the desired angle by a flexible arm. The surgeon (N.D.B.) sat at one end of the operating table. This osteosarcoma was validated by clinical studies showing lower levels of inflammatory response after a laparoscopic operation also appears less. In a comparative study of C-reactive protein levels after laparoscopic and open cholecystec-

RESULTS

EXPERIMENT A: CO₂ PNEUMOPERITONEUM VS GASLESS LAPAROSCOPY

One rat in each group died of anesthetic causes. Body weight proved to be normally distributed. No significant differences in postoperative weight loss were found between rats that had laparoscopic small-bowel resection with either CO₂ pneumoperitoneum or gasless laparoscopy (Table 1). The Figure shows the tumor growth underneath the renal capsule from day 0 (implantation) to day 3 (intervention) to day 7 (sacrifice) for each of the 4 groups. The distribution of tumor weight was statistically significant within all groups. The distribution of tumor weight was not normal, so a logarithmic transformation was applied. Significant differences in tumor growth were found between the group having CO₂ laparoscopy (group 1) and the group having gasless laparoscopy (group 2) (Table 1).

EXPERIMENT B: CO₂ PNEUMOPERITONEUM VS AIR PNEUMOPERITONEUM

Postoperative body weight loss showed a normal distribution. No significant differences in postoperative weight loss were found between the group having CO₂ pneumoperitoneum (group 3) and the group having air pneumoperitoneum (group 4) (Table 2). A logarithmic transformation was applied to analyze tumor weight because tumor weight distribution was not normal. No significant differences in tumor growth were found between laparoscopic bowel resection with CO₂ pneumoperitoneum (group 3) and air pneumoperitoneum (group 4) (Table 2).

COMMENT

Minimally invasive surgical procedures have become popular because of their favorable postoperative course. Reduced postoperative pain, early mobilization, and short hospital stays appear to result from several factors. Possibly the most important factor is reduced surgical trauma due to the use of minimal incisions and minimally traumatic operative techniques. This assumption has been validated by clinical studies showing lower levels of interleukin 6, which reflects tissue trauma, after laparoscopic compared with open surgical procedures. The inflammatory response after a laparoscopic operation also appears less. In a comparative study of C-reactive protein levels after laparoscopic and open cholecystec-
traperitoneally insufflating CO2.3 Tumor recurrences in cal treatment, concern exists about the ill effects of in-
illness was insufflated than with laparotomy.
less subcutaneous tumor growth after the abdominal cav-
cancer cells, which has been described by
mixture was described by a diffusion process, as mentioned ear-
mechanism for reducing the incidence of abdominal
anatomy, lower levels of C-reactive protein were found af-
after a laparoscopic surgical procedure.17 Oncologically,
laparoscopic surgical treatment seems advantageous, as shown in experimental studies of rats that showed less peritoneal tumor and tumor growth after laparoscopic surgical treatment.7,8 In this study, no significant differ-
ences in tumor growth were found after laparoscopic small-bowel resection and anesthesia only in the subre-
nal capsule assay, using another tumor, the CC531 co-
subcapsular tumor was measured by weighing the enucle-
data onto a logarithmic scale. Results were considered sig-
ificantly different when the mean (±SD) value of the collected data was calculated. To test for significant differences, an analysis of covariance was used.12 This analysis assumes a normal distribution, which was tested with the Kolmogorov-Smirnov test using a com-
mmercial statistical software package (SPSS Inc, Chicago, Ill). In the absence of a normal distribution, data were trans-
formed logarithmically. Nonnormally distributed data can be analyzed as if they are normally distributed by recalculating the data onto a logarithmic scale. Results were considered sig-
ificant at a 2-sided P value of less than .05.

Experiment A: CO2 Pneumoperitoneum vs Gasless Laparoscopy

On day 1, 22 rats had a 2.5-cm midline laparotomy, and 8 mg of solid ROS-1 tumor was placed under the capsule of both kidneys under microscopic vision, as mentioned ear-
two other 5-mm ports were introduced under direct vision, 1 in the upper left and 1 in the upper right quadrants of the abdomen. Rats that had a pneumoperitoneum were insufflated with CO2 or air (oxygen, 21%, and nitrogen, 79%) to a maximum pres-
sure of 6 mm Hg during 20 minutes (average total vol-
volume, 75 L). Mechanical elevation of the abdomen was est-
blished by 3 sutures that attached the trocars to a metal arm positioned over the rat. In all laparoscopic proce-
dures, a small-bowel resection (4 cm in length) followed laparoscopic extraction of an 8-cm segment of the ileum. Anastomosis was performed extracorporeally with a 7-0 silk running suture. All trocar holes were closed with a 2-0 silk suture. The resection time ranged from 25 to 35 minutes. To terminate anesthesia, 5-mg/ml atipamezole hy-
drochloride (Antisedan, SmithKline Beecham Pharmaceuticals), 2 mg/kg intramuscularly, was given.

Experiment B: CO2 Pneumoperitoneum vs Air Pneumoperitoneum

On day 1, 22 rats underwent a 2.5-cm midline lapar-
atomy, 75 L) was established by 3 sutures that attached the trocars to a metal arm positioned over the rat. In all laparoscopic proce-
dures, a small-bowel resection (4 cm in length) followed laparo-
scopic extraction of an 8-cm segment of the ileum. Anastomosis was performed extracorporeally with a 7-0 silk running suture. All trocar holes were closed with a 2-0 silk suture. The resection time ranged from 25 to 35 minutes. To terminate anesthesia, 5-mg/ml atipamezole hy-
drochloride (Antisedan, SmithKline Beecham Pharmaceuticals), 2 mg/kg intramuscularly, was given.

Decreased extraperitoneal tumor growth after lapa-
scopic surgical treatment was also reported by Allen-
dorf et al,18 who found in a murine model significantly less subcutaneous tumor growth after the abdominal cav-
ity was insufflated than with laparotomy.

Despite apparent advantages of laparoscopic surgi-
cal treatment, concern exists about the ill effects of in-
traperitoneally insufflating CO2.3 Tumor recurrences in the abdominal wall after laparoscopic resections for can-
cer have been reported by various authors.7 Although the pathogenesis of port site metastases has not been com-
pletely determined, the insufflation of gas has been sug-
gested to be an important factor. One hypothesis is that cancer cells concentrate at port sites due to the leakage of aerosolized cancer cells, which has been described by

Kazemier el et19 as the “chimney effect.” A possible mechanism for reducing the incidence of abdominal wall metastases after laparoscopic surgical therapy is to use gasless laparoscopy to prevent the spreading of tu-
mer cells by aerosolization and turbulence.20 This assu-
ption was confirmed in an experimental study of rats showing the absence of port site metastases after gasless laparoscopy.21

In this study, we attempted to assess the systemic effects of different laparoscopic techniques by scoring tu-
mor growth underneath the renal capsule. This model was used because, in contrast to peritoneal tumor mod-
els, it allows an accurate assessment of tumor growth. In these models, a quantitative analysis of tumor growth is impedied by a diffuse and disorderly growth of tumor cells. Subrenal tumor growth was less after gasless sur-
gical treatment than with CO2 pneumoperitoneum. Be-
cause subrenal tumor growth after air and CO2 insuffla-
tion was similar in this study, the increased intraperitoneal pressure may be the most important factor causing in-
creased tumor growth.

Increased intraperitoneal pressure induces a vari-
techniques. Eleftheriadis et al10 showed in an ex-
perimental study that intestinal ischemia, free oxygen rad-
cal production, and increased bacterial translocation
Carbon dioxide insufflation also causes profound hemodynamic and respiratory changes. Several studies showed that CO₂ insufflation in laparoscopic operations causes hypercarbia and fatal complications such as gas embolism, arrhythmia, or cardiac arrest. These complications stimulated the use of alternative methods of obtaining access to the abdominal cavity such as insufflating with air or mechanically elevating the abdominal wall (gasless laparoscopy). McDermott et al. showed that CO₂ insufflation, unlike gasless laparoscopy, led to a fall in partial blood oxygen pressures and the absorption of CO₂, resulting in hypercarbia, acidosis, and consequent hyperdynamic circulation. Davidson et al. showed that gasless laparoscopy may provide a safer method of exposure than CO₂ insufflation for minimally invasive surgical procedures in patients with preexisting pulmonary or cardiac dysfunction. Whether intraperitoneal CO₂ insufflation, directly or indirectly by pH, hypercarbia, or increased intraperitoneal pressure, affects tumor growth remains unknown.

To evaluate the effect of CO₂ toxicity on metabolism, we compared the postoperative body weight in rats having either CO₂ or air pneumoperitoneum. In this experiment, the postoperative restoration of body weight was not different in the groups undergoing CO₂ and air pneumoperitoneum. To eliminate possible metabolic stress due to an elevated intraperitoneal pressure, the postoperative body weight was also assessed in rats having either CO₂ pneumoperitoneum or gasless laparoscopy. Differences were also not found in this experiment. Apparently the adverse hemodynamic, respiratory, and hormonal effects of CO₂ insufflation did not affect the rate of the postoperative recovery of body weight. Body weight is an indication of the entire complex of metabolic processes. Extrapolating this observation to the clinical situation should be done with care because the duration of the exposure of the peritoneal cavity to gas insufflation in this study was relatively short compared with that in clinical practice. Furthermore, the postoperative body-weight observation period in this experiment was short, only 5 days. The apparent difference in increases in tumor weight between groups 1 and 3 is probably due to the use of different batches of ROS-1 tumor cells that were used in experiments A and B.

Although variables such as intraperitoneal pressure and operative time differed in this study from those in daily clinical practice, gasless laparoscopic bowel resection was associated with less tumor growth under-
neath the renal capsule compared with \( \text{CO}_2 \) and air insufflation. Therefore, the feasibility of the application of gasless laparoscopy deserves further evaluation. The pathophysiological effects of intraperitoneal insufflation with either \( \text{CO}_2 \) or air in abdominal cancer are unclear and merit extensive study.

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REFERENCES