Videoendoscopic Endotracheal Intubation in the Rat: A Comprehensive Rodent Model of Laparoscopic Surgery1, 2

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Background. Peritoneal absorption of CO2 during abdominal insufflation in laparoscopy may disrupt the acid–base equilibrium and alter the physiological response to stress. Current nonventilated rodent models of laparoscopy do not manage the CO2 load of pneumoperitoneum, but ventilated surgical rodent models are invasive (tracheotomy) and may independently induce the inflammatory response.

Materials and methods. A comprehensive rodent model of laparoscopy was developed. Rats were randomized to receive anesthesia alone, anesthesia plus CO2 pneumoperitoneum, or anesthesia plus CO2 pneumoperitoneum with videoendoscopic intubation and mechanical ventilation. Arterial blood-gas analysis was performed at baseline and after 30 min of intervention.

Results. Baseline pH, pCO2, and HCO3− arterial blood gas parameters were normal for all rats. After 30 min, pCO2 and pH changed slightly but remained normal among rats receiving anesthesia alone (pCO2 = 46.5 ± 1.9; pH = 7.365 ± 0.009) whereas animals receiving anesthesia plus CO2 pneumoperitoneum that were dependent on spontaneous respiration for ventilation developed significant hypercarbic acidosis (pCO2 = 53.2 ± 1.9, P < 0.05; pH = 7.299 ± 0.011, P < 0.001). This acidosis was completely corrected with increased minute ventilation in intubated rats receiving mechanical ventilation (pCO2 = 36.8 ± 1.5, P < 0.001; pH = 7.398 ± 0.011, P < 0.001).

Conclusions. CO2 pneumoperitoneum induces significant hypercarbic acidosis in nonventilated rats. Noninvasive endotracheal intubation is feasible in the rat with videoendoscopic assistance. Our noninvasive rodent model of laparoscopic surgery controls for anesthesia- and capnoperitoneum-related acid–base changes and provides an environment in which the biological response to pneumoperitoneum can be studied precisely.

Key Words: carbon dioxide; CO2; laparoscopy; pneumoperitoneum; animal models; rats; acidosis; intubation; endotracheal intubation; minimally invasive surgical procedures.

INTRODUCTION

As the principles of minimally invasive surgery have gained acceptance throughout the surgical community during the past two decades, laparoscopy has revolutionized the field of general surgery. Since the first laparoscopic cholecystectomy was performed in 1985, laparoscopic surgery has become preferred over open surgery for a variety of surgical conditions. The advantages of laparoscopic surgery over conventional surgery include decreased postoperative pain, decreased tissue trauma, shorter length of stay, faster recovery, more rapid return to oral intake, and less adhesion formation [1–5]. Although a great deal has been learned about the advantages of minimal access surgery, the tremendous potential this methodology has to offer is only beginning to be appreciated [6–9].

Many of the hemodynamic, immunological and hormonal effects of capnoperitoneum have been well characterized in both animal and human studies [10–19]. CO2 pneumoperitoneum has been implicated in the release of cortisol, epinephrine, norepinephrine, renin, prostaglandins, and vasopressin, all leading to in-
creases in systemic vascular resistance [16, 18]. Regarding the immunological effects of laparoscopy, a number of studies from our group and others indicate that CO₂ pneumoperitoneum may alter the immunological profile in surgical patients [20–25]. The common denominator between the changes observed within these different physiological systems is the CO₂ pneumoperitoneum. To study the plethora of physiological differences between minimally invasive and conventional surgery, laparoscopy is becoming increasingly important in experimental animal models.

One of the main physiological perturbations that can occur during laparoscopic surgery is hypercapnic acidosis induced by the absorption of carbon dioxide during pneumoperitoneum. Although the direct depressive effects of acidemia on the myocardium may predominate over the indirect effects of hypercapnic stimulation of the autonomic nervous system [26], a number of studies have shown that hypercapnia has mixed effects on cardiac function [27–30]. This notion is consistent with studies in laparoscopy that show that CO₂ pneumoperitoneum leads to a reduction in stroke volume, tachycardia, and normal cardiac index with increased systemic and pulmonary vascular resistance. This is just one example. In fact, because of the pH sensitivity of many enzymatic functions, virtually every biological system is affected in some fashion by changes in systemic acid–base status. However, current animal studies do not always reflect the clinical conditions that are being “modeled.” This is especially true in studies of laparoscopy in which animals are not mechanically ventilated.

In this work we address the magnitude of the effects of CO₂ pneumoperitoneum on acid–base status in anesthetized, nonventilated models of laparoscopic surgery. Furthermore, we have developed a comprehensive model for laparoscopic surgery in the rat that controls for CO₂ pneumoperitoneum-induced hypercapnic acidosis. We submit our experience with rodent models of laparoscopy anticipating that our recommendations will enable investigators to improve experimental planning for rodent models of laparoscopy.

MATERIALS AND METHODS

Effects of CO₂ Pneumoperitoneum on the Acid–Base Equilibrium

In this article, 35 rats were randomized into three groups: 1) anesthesia alone (control, n = 15); 2) anesthesia plus carbon dioxide pneumoperitoneum (n = 10); and 3) anesthesia plus carbon dioxide pneumoperitoneum with videodendoscopic intubation and mechanical ventilation (n = 10). Baseline and 30-min arterial blood gas samples were collected from each animal through a femoral arterial line.

Animal Care

Adult male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing approximately 250–350 g were housed in plastic cages where standard chow and water were available ad libitum. Animals were acclimatized to their environment for 3–5 days after arrival and were then fasted for 16 h prior to procedures. The animal housing environment maintained a 12-h light/dark cycle, a temperature of 72°F, and a humidity ranging between 30% and 70%. After completion of the experiment, all rats were euthanized via anesthetic overdose. All animal procedures were performed on a protocol approved by Johns Hopkins Medical Institutions Animal Care and Use Committee (IACUC; in this case, Protocol #RA01M445).

Videodendoscopy-Assisted Endotracheal Intubation

Before intubation, rats were preanesthetized rapidly in a 15 × 15-inch glass jar using vaporized isoflurane (“IsoFlo” Abbot Laboratories, North Chicago, IL). Spontaneously breathing anesthetized animals were placed supine on a 20 × 15 cm Styrofoam board and restrained with adhesive tape (Fig. 1). Anesthesia was maintained by delivering 3% of vaporized isoflurane thru a nose cone. With the neck extended, oppositional retraction of the superior and inferior incisor teeth was achieved using 1-cm “teeth loops” made from 3-O silk suture that were fixed to the Styrofoam board with needles (Fig. 2A). Use of this simple combination of Styrofoam, suture, and needles affords an inexpensive, completely adjustable rodent retraction system (useful for much more than jaw retraction). Inferior and lateral retraction of the tongue was achieved using a penetrating towel clamp. A rigid 30-degree 3-mm endoscope (generously donated by Karl Storz Endoscopy, Germany) was used to visualize the epiglottis, larynx, and vocal cords viewed on the monitor (Fig. 2B). Using its previously filed, blunted and bent metal insertion needle as a stylet, a 14-gauge intravenous catheter was inserted through the mouth in parallel with the scope. The catheter was carefully advanced between the vocal cords and into the trachea (Fig. 2C). The stylet was then removed and the catheter was secured to the snout with tape. The catheter was connected to a Harvard Rodent ventilator model 683, and during procedures requiring CO₂ pneumoperitoneum, ventilated with a min ventilation of approximately 850 ml/kg/min (approximately 250 ml/min for a 250–350 g rat).

Arterial Line Insertion

The right groin of the rat was shaved and prepped with 70% isopropyl alcohol. The lower extremities were taped in extension, and a 1-cm groin incision was made over the palpated pulse of the femoral artery (Fig. 3A). Intubation was conducted down to the muscular layer until femoral neurovascular complex was identified. A 1-cm segment of the femoral artery was bluntly dissected free from the vein, nerve, and associated connective tissue. A 3-O silk ligature was used to ligate the distal end of the isolated portion of artery. This suture was then used to apply gentle retraction along the axis of the extremity to straighten the vessel in preparation for catheterization. A second 3-O silk ligature was “looped” around the proximal portion of the isolated artery and a single throw “air knot” was made but not cinched. A small lightweight forceps was used to “tag” this second ligature and was draped over the animal such that the weight of the clamp produces slight upward traction on the vessel, thus collapsing the opposing vessel wall and preventing hemorrhage when the arteriotomy was performed. Castroviejo microscissors were used to make a small arteriotomy (approximately 30% of the circumference perpendicular to the axis of the vessel) between the distal ligation and the proximal loop (Fig. 3B). A mini catheter introducer made from 25-gauge needle with the tip bent backward on itself was used to lift up the top arterial wall, and the beveled end of an arterial catheter made from polyethylene tubing with an outer diameter of 0.965 mm and an internal diameter of 0.58 mm flushed with heparinized saline was gently introduced approximately 1 cm into the artery (Fig. 3C). The “air knot” of the proximal ligature was then slid down over the catheterized portion of the vessel and was tied, thus securing the catheter in the vessel.
Laparoscopic Techniques

The abdominal wall was shaved and prepped with 70% isopropyl alcohol. Insufflation was achieved using a laparoscopic insufflator (Olympus) connected to a fiberoptic light source and video camera (Olympus America Inc., Melville, NY) to visualize the peritoneal cavity on a 13-inch color video monitor (Olympus). We use six Kleinsasser endolaryngeal microsurgery instruments (Storz): two straight micro grasping forceps, one curved micro grasping forceps, one micro cupped jaw forceps, one miniature scissors, and one needle holder. All instruments have a working length of 20 cm and are introduced directly through small holes in the abdominal wall (without trochars) created by puncture with a 14-gauge angiocatheter. The holes are small enough to allow maintenance of the pneumoperitoneum.

Experimental Set-up Special Considerations

In our comprehensive model, the ideal gas for rodent laparoscopic surgery is carbon dioxide because of its properties of being odorless, colorless, stable, buffered in the blood, eliminated by the lungs, having a low risk of venous gas embolism, and being nonflammable/nonexplosive. Furthermore, CO₂ pneumoperitoneum has been shown to have beneficial immune-modulating effects [25, 31]. The amount of pneumostatic pressure that should be used to insufflate the rat abdomen warrants particular consideration. There is increasing evidence in the literature that maintaining the lowest possible pressure to create the CO₂ pneumoperitoneum is the best option, mainly because fewer deleterious hemodynamics changes occur at lower pressures [32–40]. In our experience, pressures of 3–4 mmHg are ideal for performing complex laparoscopic surgical tasks in the rat. Also of note is the importance of fluid support: we use a 10-ml/kg bolus of 0.9% NaCl administered subcutaneously in the scruff of the neck in the immediate post-operative period. Maintaining body temperature with a heating device such as a simple heat lamp or more complex forced air warming devices is important to prevent hypothermia. Hypothermia increases both morbidity and mortality under critical conditions, and can have significant effects on gross and molecular physiology [41–46].

Description of Statistical Methods

The one-way analysis of variance test was used to detect general differences in arterial blood gas parameters between groups. To elucidate specific significances in these parameters between groups, multiple pairwise comparisons were performed using Tukey’s test. All results were presented as the mean ± the standard error of the mean. Differences between groups were considered significant when P < 0.05. Analysis was performed using SigmaStat 3.0 (SPSS Inc., Chicago, IL) software.

RESULTS

The learning curve associated with videoendoscopy-assisted endotracheal intubation was not insignificant. After trying this procedure on 10–15 “practice” animals, the overall success rate for operators in our laboratory was between 80% and 90%. The “failures” were caused largely by the fact that animals with even relatively mild iatrogenic laryngeal trauma had significant respiratory difficulty postoperatively. Thus, because our laboratory also performs survival surgery using this model, we limit the intubation process to three attempts. Difficulties with intubation are primarily related to animal movement due to laryngeal stimulation in unparalyzed animals. For this reason, the concentration of vaporized isoflurane was in-
creased (4–5%) briefly immediately prior to intubation. In this study, two failed intubation attempts occurred. These rats were replaced with new animals.

The learning curve associated with our method of arterial cannulation plateaus much more quickly, and after 10-15 “practice” animals, the operators in our laboratory achieve a near-100% success rate. All 35 femoral artery catheterization attempts were successful in this study. All attempts at creating a pneumoperitoneum in this study were successful as well.

Arterial blood gas parameters were measured in rats at baseline and after 30 min of anesthesia alone, anesthesia plus carbon dioxide pneumoperitoneum, or anesthesia plus carbon dioxide pneumoperitoneum with mechanical ventilation. Animal weight (mean = 317 g), intraabdominal insufflation pressure was 4 mmHg, and the total duration of all procedures (mean = 118 min) were comparable between groups. Baseline pH, pCO₂, and HCO₃⁻ arterial blood gas parameters were normal for all rats (Table 1). After 30 min of anesthesia alone pCO₂ increased slightly (from 42.6 ± 1.1 to 46.5 ± 1.9) and pH decreased slightly (from 7.399 ± 0.009 to 7.365 ± 0.009) thus reflecting the mild respiratory suppressive effects of isoflurane anesthesia. After 30 min of anesthesia and CO₂ pneumoperitoneum, rats dependent on spontaneous respiration for ventilation developed significant hypercarbic acidosis with a mean pCO₂ of 53.2 ± 1.9 (P < 0.05 versus anesthesia alone) and a mean pH of 7.299 ± 0.011 (P < 0.001 versus anesthesia alone). This acidosis was completely corrected with increased min ventilation in the intubated rats receiving mechanical ventilation (mean pCO₂ = 36.8 ± 1.5, mean pH = 7.398 ± 0.011, P < 0.001 for both versus spontaneous ventilation anesthesia + CO₂ pneumoperitoneum).

**DISCUSSION**

Laparoscopic surgery has become the gold standard for a variety of surgical indications [8], with laparoscopic cholecystectomy being the most widely and rapidly accepted throughout the world [47–51]. Other organs routinely being approached via the laparoscope include the adrenal glands [52], the esophagus for antireflux surgery [53–56], and the spleen [57, 58] to mention a few. Continued advancement of the field of laparoscopic surgery depends on the use of animal models. The rodent model of laparoscopy is being applied to investigations spanning the spectrum from clinical to basic science. Everything from enteric resection and anastomosis, gastric bypass, splenectomy, and nephrectomy [59] to surgically- and endotoxin-induced hypercarbic acidosis.
models of sepsis is being performed in rats and mice applying the laparoscopic paradigm. Only a few publications describe the use of such models for specific minimal access surgery applications [60, 61].

We have developed an effective rodent model of minimally invasive abdominal surgery that we anticipate will serve as a guide for investigators in the surgical research community interested in studying the physiology, immunology, and outcomes unique to the laparoscopic approach. This comprehensive laparoscopic

TABLE 1

Arterial Blood Gas Parameters before and after Intervention Among Rats Receiving: Anesthesia Only, CO₂ Pneumoperitoneum, or CO₂ with Mechanical Ventilation

<table>
<thead>
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<th>Baseline</th>
<th>After 30 min</th>
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<tbody>
<tr>
<td></td>
<td>pH</td>
<td>pCO₂</td>
</tr>
<tr>
<td>Anesthesia</td>
<td>7.399 ± 0.009</td>
<td>42.6 ± 1.1</td>
</tr>
<tr>
<td>CO₂ Pneum</td>
<td>7.409 ± 0.006</td>
<td>39.3 ± 0.9</td>
</tr>
<tr>
<td>CO₂ Pneum + Vent</td>
<td>7.419 ± 0.017</td>
<td>38.2 ± 2.5</td>
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Note. Values expressed as mean ± SEM (n = 15 for anesthesia group, n = 10 for CO₂ Pneum and CO₂ Pneum + Vent groups). Statistical analysis via one-way ANOVA (analysis of variance).

* P < 0.001 vs. both Anesthesia pH and CO₂ Pneum + Vent pH.
† P < 0.05 vs. Anesthesia pCO₂ and P < 0.001 vs. CO₂ Pneum + Vent pCO₂ after 30 min.
surgery rat model addresses important issues such as anesthesia- and pneumoperitoneum-induced acid–base effects, circulatory system access and fluid support, pneumoperitoneum insufflation pressure and gas, and choice of anesthetic agents.

Because of the ease of using anesthetic regimens that do not cause severe respiratory suppression in rodent experimentation, mechanical ventilation is not performed in the majority of animal surgical experiments. Although a number of minimally invasive techniques for gaining airway access in rodents have been described, tracheotomy is used most commonly. This method is excellent for nonsurvival experiments and for studies in which the addition of the tracheotomy would likely not directly affect the outcome. But because those investigating laparoscopy are concerned about the invasiveness of their surgery, these researchers usually choose to avoid tracheotomy. Furthermore, because many of the noninvasive means of intubation in rodents require specialized skill and/or equipment to employ, the majority of laparoscopy researchers elect not to mechanically ventilate their animals at all, but rather depend on anesthetic regimens believed to have minimal respiratory suppressive effects.

Our findings corroborate those of other investigators indicating that carbon dioxide pneumoperitoneum can have profound effects on acid–base equilibrium in non-ventilated animals [30, 62, 63]. We found that after only 30 min of abdominal insufflation with CO₂, rats anesthetized with an agent touted as possessing only mild effects on respiratory drive at low doses (isoflurane) develop statistically and clinically significant accumulation of CO₂ (pCO₂ = 53.2) and acidosis (pH = 7.299). We have shown that adding endotracheal intubation with an appropriate min ventilation restores acid–base equilibrium completely (pCO₂ = 36.8, pH = 7.398). It is our concern that, because the enzymatic function of many proteins can be profoundly affected by changes in pH, models that allow the accumulation of CO₂ produce an environment that may influence outcomes and are thus not representative of the clinical scenarios that these models are intended to mimic. Mechanical ventilation should be considered in laparoscopy experimentation whenever it is possible that systemic acid–base balance could affect the results of the experiment.

In the current study we have demonstrated that endotracheal intubation can be performed in the rat by a single operator with videendoscopic assistance. When animals are deeply anesthetized or sedated, this method consistently provides excellent exposure of the vocal cords, thus making the technique relatively easy to learn. A number of other methods for endotracheal intubation in rodents have been described in the literature. Rigid cannula intubation was first reported by Jaffe and Free in 1973 [64]. Pena and Cabrera reported improved visualization of the vocal cords by enlisting the aid of a surgical microscope [65], and Alpert et al. reported a simple means of direct laryngoscopy emphasizing the importance of a good light source [66]. Although rarely used today, Stark et al. described a method for blind intubation [67]. Weksler et al. reported intubation using an otoscope [68], and Linden et al. described the use of a modified laryngoscope to intubated rats [69]. Worthley et al. were the first to report use of a fiber-optic scope to aid in the intubation of rabbits [70]. Recently, Vergari et al. reported intubation in mice using arthroscopic equipment very similar to that used in our laboratory [71]. The fact that so many different methods for rodent intubation have been described probably indicates that there is no “best” way. Our method of using our rigid 3-mm laparoscope as a laryngoscope works well and provides our laboratory the additional advantage of being cost-effective. By leveraging previously acquired videendoscopic equipment, laparoscopic surgery researchers can improve the validity of their experiments at very low cost.

In the current study, femoral arterial catheters were placed to allow measurement of arterial blood-gas parameters. In our model in general, the placement of a femoral arterial line also facilitates blood sampling at different time points during kinetic studies and measurement of hemodynamic parameters. Our experience in using this model in survival experiments has shown that when the femoral nerve is uninjured during the arterial dissection, rats have a fully functional limb post-operatively with no evidence of cyanosis. Therefore, investigators using this technique do not need to be overly concerned about distal limb ischemia when ligating the rat femoral artery. For studies that necessitate repeated intravenous drug delivery or blood sampling over a number of weeks, implantable catheters with subcutaneous access ports are a good option. Numerous investigators have reported on the use of these devices, and some have used them for experiments lasting over a month [72, 73]. In our experience, placement of a femoral arterial catheter in a 250- to 350-g rat takes between 3 and 5 min among individuals who have mastered the technique during the course of 10–14 attempts (5–7 rats). A few technical points discovered through our experience with this simple but delicate procedure may be of interest to the investigator who plans to use this model: 1) We have found that dipping the tip of the catheter in mineral oil eases the insertion of the catheter a great deal. 2) Applying gentle steady tension on the vessel by retracting (along the axis of the limb) the distally ligated artery using its 3-0 silk suture facilitates a simple and atraumatic catheter tip insertion. 3) The use of a homemade “catheter introducer” (described earlier) placed through the arteriotoomy greatly facilitates the opening of the vessel lu-
men. 4) Minimizing the length of vessel dissection to 1 cm or less prevents the artery from being a “moving target” as the connective tissue stabilizes the vessel.

We limited blood-gas analysis to that of the arterial blood in both spontaneously breathing and mechanically ventilated rats undergoing CO₂ pneumoperitoneum. Kuntz et al. studied in rats the pH in three different compartments (arterial blood, subcutaneous fat tissue, and intra-abdominal) during CO₂ pneumoperitoneum in spontaneously breathing animals only, and found that pressures above 6 mmHg had dramatic pH changes in all compartments, while pressures below this level had minimal changes in all three compartments [74]. Future experiments should evaluate such parameters in the portal system where one might expect an even greater degree of acidemia from absorbed CO₂ that persists to some degree even after the systemic arterial pH has been corrected via mechanical ventilation.

In summary, we have demonstrated that noninvasive endotracheal intubation is feasible and beneficial in the rat with videoendoscopic assistance. Furthermore, our study confirms that CO₂ pneumoperitoneum induces significant hypercarbic acidosis in non-ventilated rats, but study confirms that CO₂ pneumoperitoneum induces significant hypercarbic acidosis in non-ventilated rats, but

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REFERENCES


