Changes in End-Tidal Carbon Dioxide due to Gastric Perforation during Pneumoperitoneum in the Rat

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ABSTRACT:

Background: Carbon dioxide is the most widely used gas to establish pneumoperitoneum during laparoscopic surgery. Gastrointestinal trauma may occur during the peritoneal insufflation or during the operative phase itself. Early diagnosis of these injuries is critical.

Objectives: To assess changes in end-tidal carbon dioxide (E_TCO_2) following gastric perforation during pneumoperitoneum in the rat.

Methods: Wistar rats were anesthetized, tracheotomized and mechanically ventilated with fixed minute volume. Each animal underwent a 1 cm abdominal longitudinal incision. A 0.3 x 0.3 cm cross-incision of the stomach was performed in the perforation group but not in the controls (n=10/group) and the abdomen was closed in both groups. After stabilization, CO_2-induced pneumoperitoneum was established at 0, 5, 8 and 12 mmHg for 20 min periods consecutively, each followed by complete pressure relief for 5 min.

Results: Ventilatory pressure increased in both groups when pneumoperitoneal pressure ≥ 5 mmHg was applied, but more so in the perforated stomach group ($P = 0.003$). E_TCO_2 increased in both groups during the experiment, but less so in the perforated group ($P = 0.04$). It then returned to near baseline values during pressure annulation in all perforated animals but only following the 0 and 5 mmHg periods in the controls.

Conclusions: When subjected to pneumoperitoneum, E_TCO_2 was lower in rats with a perforated stomach than in those with an intact stomach. An abrupt decrease in E_TCO_2 during laparoscopy may signal gastric perforation.

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KEY WORDS: pneumoperitoneum, end-tidal carbon dioxide (E_TCO_2), stomach, perforation

C arbon dioxide is the most widely used gas to establish pneumoperitoneum during laparoscopic surgery. It is non-combustible, inexpensive and the least emboligenic [1,2]. Intraabdominal CO_2 is absorbed through the peritoneal surface and vasculature into the bloodstream and then eliminated via the expired air [3,4]. This leads to an increase in the measured end-tidal CO_2, which is proportionate to the alveolar CO_2 partial pressure. Both values are likely to increase (hypercarbia) during laparoscopic surgery if minute ventilation is not adjusted properly [5,6].

Gastric perforation during laparoscopic surgery is an infrequent event [7]. Gastrointestinal trauma may occur during peritoneal insufflation or during the surgery itself. Based on data provided by van der Voort and colleagues [7], the most common location of injury is the small bowel (55.8%), followed by the large intestine (38.6%) and, less commonly, the stomach (3.9%). Early diagnosis of these injuries is critical to prevent morbidity and mortality associated with viscus perforation.

We are not aware of published studies describing changes in E_TCO_2 when the stomach is deliberately or inadvertently perforated during laparoscopic surgery. We reported previously that small bowel perforation during laparoscopy was associated with an immediate increase in E_TCO_2 [8]. In the present study we investigated whether similar gas changes could occur if the stomach had been perforated while the peritoneal cavity was pressurized (pneumoperitoneum).

MATERIALS AND METHODS

Twenty adult male Wistar rats (weight 350–450 g) were kept in accordance with the guidelines of the Committee on Animal Research at the Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, and approval for the study was obtained.

SURGICAL PREPARATIONS AND PROCEDURES

All animals were anesthetized with intraperitoneal ketamine 5 mg/kg and diazepam 1 mg/kg. A tracheostomy was then performed and the rats were mechanically ventilated with room air, using a piston-type rodent ventilator set to deliver 10 ml/kg body weight tidal volume at 45 breaths/minute; this resulted in E_TCO_2 of 26–30 mmHg. Positive end-expiratory pressure was maintained at 4 mmHg. The right femoral artery was cannulated with a 20 gauge catheter for continuous invasive blood pressure and heart rate monitoring. E_TCO_2 was continuously

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E_TCO_2 = end-tidal carbon dioxide

monitored (Capnomac Ultima®, Datex, Helsinki, Finland) and recorded, as was peak ventilatory pressure.
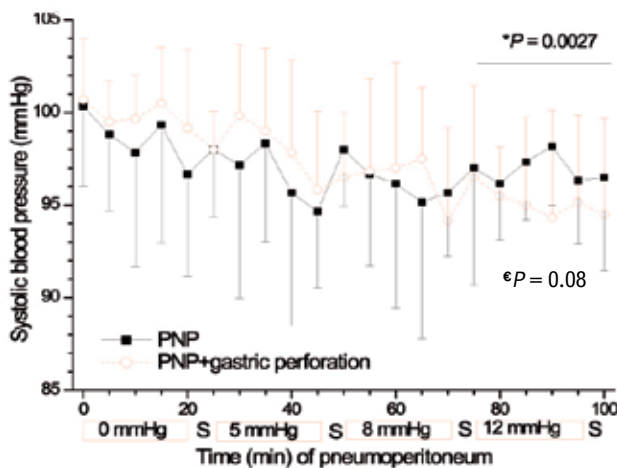
EXPERIMENTAL PROTOCOL

The rats were divided into two groups (n=10 per group): pneumoperitoneum (control) and pneumoperitoneum plus perforation. After all the cannulations were performed the rats were allowed to stabilize (20 min). The stomachs were then emptied by gentle suction via a neonatal tube and pH was measured in three animals in each group. In the PNP+P group a 1 cm longitudinal laparotomy was performed followed by a 0.3 x 0.3 cm cross-incision of the stomach. The abdominal wall was sutured with 2/0 vicryl and the animal was left to re-stabilize for 20 min. The PNP animals underwent the same laparotomy but the stomach was left intact.

The abdominal cavity was pressurized at different levels gradually in both groups [9]: each animal was exposed to intraabdominal pressure for 20 min periods at 0, 5, 8 and 12 mmHg each; each pressure period was followed by a complete pressure-relief interval of 5 min. At the end of the experiments the animals were euthanized and the presence of gastric perforation in each group was ascertained. The degree of acidity or alkalinity of the stomach measured both before and at the end of the experiment was determined by using the IQ150 pH meter (Meter Spectrum Technologies, Inc., IL, USA). This device permits instant and accurate measurement of pH in soil media as well as in water or nutrient solutions.

PNP+P = pneumoperitoneum plus perforation
PNP = pneumoperitoneum

STATISTICAL ANALYSIS

Data were summarized as mean ± standard deviation. Differences in variables were analyzed by Wilcoxon signed rank test; $P \leq 0.05$ was considered statistically significant.
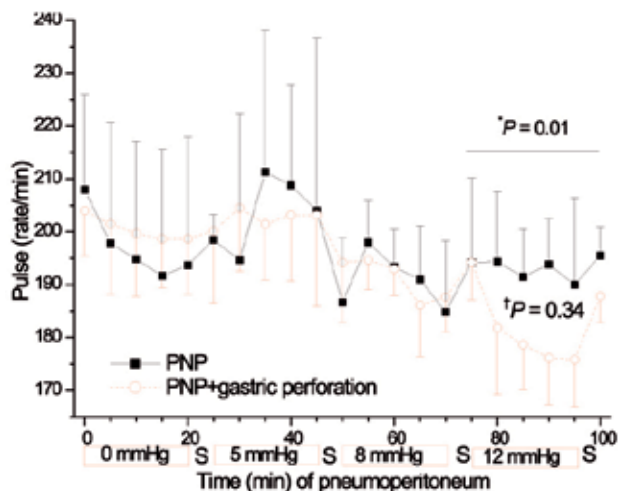
RESULTS

Stomach perforation was still patent at the end of the experiments in all PNP+P animals. The pre-experimental pH ranges were similar in all rats (6.1–6.5); the mean end-experimental pH in the perforated stomachs was significantly lower than in the non-perforated, 5.17 ± 0.289 vs. 5.87 ± 0.11 ($P = 0.0041$).

There were no significant differences in systolic blood pressure throughout the experiments in both groups, except for the 12 mmHg pressure period when it was lower in the PNP+P group ($P = 0.0027$) [Figure 1]. Heart rate was higher in the PNP compared to the PNP+P group during the same pressure period ($P = 0.01$) [Figure 2].

Ventilatory pressure increased in both groups when ≥ 5 mmHg pressure was applied intraperitoneally, more so in the PNP+P group ($P = 0.003$) [Figure 3].

E_TCO_2, which increased in both groups during the pressure insufflation periods, was more pronounced in the PNP group ($P = 0.04$). Furthermore, unlike the PNP+P animals, where E_TCO_2 returned to near-baseline pressure values when any pressure gradient was halted, it did not decrease in the PNP animals when the 8 and 12 mmHg intraperitoneal pressures decreased to zero [Figure 4].

Figure 1. Changes in systolic blood pressure between the groups

*P = 0.0027 between the groups when subjected to 12 mmHg pneumoperitoneal pressure
€P = 0.08 between the groups throughout the experiment
S = 5 minutes of peritoneal pressure relief and stabilization
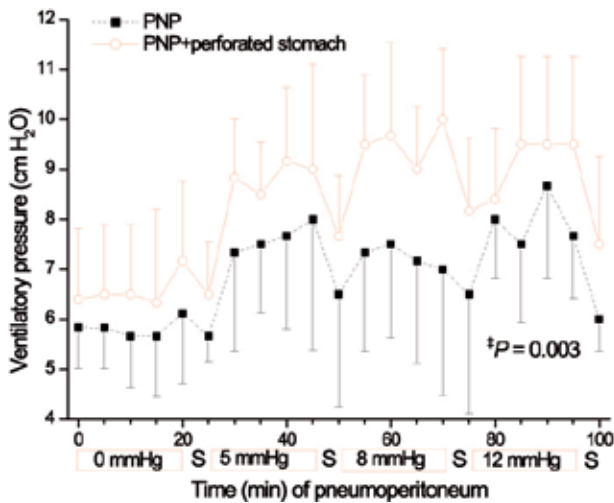
Figure 2. Changes in pulse rate

*P = 0.01 between the groups when subjected to 12 mmHg pneumoperitoneal pressure
†P = 0.34 between the groups throughout the experiment
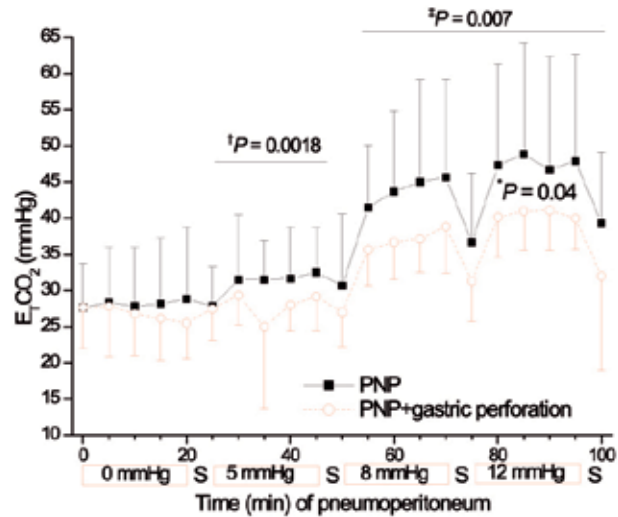S = 5 minutes of peritoneal pressure relief and stabilization

Figure 3. Changes in ventilatory pressure

‡$P = 0.003$ between the groups throughout the experiment, with exception of 0 mmHg pressure

S = 5 minutes of peritoneal pressure relief and stabilization

Figure 4. Changes in end-tidal carbon dioxide (E_TCO_2)

*$P = 0.04$ between the groups throughout the experiment

†$P = 0.0018$ between the groups during the 5 mmHg pressure phase

‡$P = 0.007$ between the groups during the 8 and 12 mmHg pressure phase

S = 5 minutes of peritoneal pressure relief and stabilization

DISCUSSION

This study, which we believe to be the first of its kind, emphasizes the end-tidal CO_2 (E_TCO_2) change during pneumoperitoneum. We demonstrated increased values in parallel with the pneumoperitoneal pressure, as had been shown earlier [8,10]. The presence of gastric perforation was, however, associated with a more attenuated rise in E_TCO_2, and with its return to baseline values even after high intraperitoneal pressure when it was decreased to zero. Contrarily, in the absence of the perforation, E_TCO_2 remained constantly high, even during the pauses in intraabdominal pressure. At the same time, VP, which usually increases during pneumoperitoneum, increased more significantly in the stomach-perforated animals. The values of E_TCO_2 and VP seemed to complement each other and represent two major signs for gastric perforation during pneumoperitoneum.

E_TCO_2 is usually a reflection of metabolism, circulation and ventilation, and parallels arterial CO_2 if there is no ventilation-perfusion mismatch. The shift from normal values derives from a high load of CO_2 in the body when insufflated into the peritoneal cavity at pressures that create a tension gradient between the pneumoperitoneum and the blood perfusing the peritoneum. Minute ventilation adjusted for E_TCO_2 usually compensates for the hypercarbia that is built up during pneumoperitoneum [1,2]. This physiological feature was the basis of our current animal study and is relevant to clinical practice.

VP = ventilatory pressure

Carbon dioxide is also involved in the synthesis of hydrochloric acid in the parietal cells of the stomach, where secretion of hydrogen ion requires the presence of carbonic acid that derives from the hydration of CO_2 [4,5]. Baraka et al. [11] demonstrated that in patients undergoing laparoscopic cholecystectomy, CO_2 insufflation is followed by a significant increase in E_TCO_2 and a significant decrease in gastric fluid pH. It was shown that such changes are inversely related to blood pCO_2 (and therefore E_TCO_2) [12]. We postulate that the decreased E_TCO_2 values detected in the PNP+P rats were due, at least in part, to the presence of gastric perforation, which allowed for abundant passage of CO_2 into the stomach where it came into direct contact with the gastric mucosa and thereby metabolized, leading to a reduced amount of E_TCO_2 washout. Indeed, the reduction in the final pH did not occur in the PNP animals, because of little or no direct exposure of the gastric cells to the gas, which supports this mechanism of CO_2 utilization by the gastric mucosa in the perforated stomach.

The perforated stomach could reduce E_TCO_2 via a mechanical mode as well. The stomach, being open both cephalically and caudally, would enable the pressurized CO_2 leak through the esophagus and the mouth upwards so that less E_TCO_2 could enter the circulation. It was not possible in the present study to measure CO_2 at the level of the buccal aperture. In the stomach, there is a possible mechanical leakage of the gas through the lower esophageal sphincter (minimal normal pressure approximating 9 cm H_2O) [13] and the mouth, evidently at the higher pneumoperitoneal pressures.

Unlike the occurrences in the perforated small bowel, perforation of the stomach leads to a lower E_TCO_2 value. In our previously reported perforated bowel model that was exposed to pneumoperitoneum [8], the relatively higher E_TCO_2 was attributed to the high rate of absorption of the gas by the blood via the rich vasculature, both in the small intestine and in the peritoneum. The stomach is much poorer in superficial vasculature than the intestine [12]. Silva et al. [14] demonstrated that CO_2 was eliminated from the small bowel lumen entirely within 15 minutes of gas insufflation. These findings support our suggestion of different measurable levels of E_TCO_2 following perforation in various intraabdominal hollow viscuses.

Finally, the pneumoperitoneal model that we used, i.e., consecutive increasing pressure gradients, further supports the above mechanistic contention of possible gas escape during high pneumoperitoneal pressures. When pressure was removed, at intervals, and the intraabdominal CO_2 pressure declined, the intragastric pressure that allowed for its escape through the esophagus was still present in the PNP+P group but not in the PNP animals. This explains why E_TCO_2 in the former appeared lower and close to baseline pressure values during pressure interruption (continuous escape) but was still high in the control group (no escape).

Ventilatory pressure was higher in the perforated group, especially when ≥ 5 mmHg pneumoperitoneal pressure was instituted. This finding also supports our assertion above, that part of the gas could escape via the esophagus and mouth when pressure gradient (pneumoperitoneal vs. oral pressures) forced the lower esophageal sphincter to partially open and allowed for a CO_2 reflux-like mechanism. When the pneumoperitoneal pressure was low, the pressure within the stomach did not allow much gas to escape cephalically, thus obstructing the small airways which caused VP to increase. Thus, the stomach constituted a pressurized area at pressures similar to those in the peritoneum, becoming an abnormal source of adjacent pressure to the lungs, leading to an increase in ventilator pressure. In the PNP (non-perforated) group, there was no intragastric pressure build-up, i.e., the factor that could obstruct airways and alveoli from being ventilated was lacking, resulting in less increased VP.

Elevated blood pCO_2 directly causes myocardial depression and vasodilation. These effects are counteracted by the CO_2 centrally mediated sympathetic stimulation that induces tachycardia and systemic vasoconstriction [15]. In our study, a lower heart rate was recorded in the 12 mmHg pressurized PNP+P animals compared to the PNP ones. This correlates with the lower blood pCO_2 in the PNP+P animals, probably due to the previously discussed escape, and a meaningful reduction of gas absorbed into the bloodstream. In the PNP rats without perforation the high intraabdominally contained pressure severely compressed the regional circulation, decreasing the blood volume that returns to the heart and,

consequently, lowering cardiac output and blood pressure. Such an event would accentuate sympathetic activity, increasing the heart rate. The minor obstruction to circulation and the higher rate of CO_2 escape and its absorption within the stomach in the PNP+P group led to lower sympathetic activity and, consequently, subtle cardiovascular stimulation.

Although we do not intend to extrapolate conclusions from this preclinical experiment to clinical practice, our data provide interesting and useful information for both the surgeon and the anesthesiologist performing laparoscopic surgery involving mainly upper abdominal organs. The anesthesiologist should be alert when the E_TCO_2 profile or values change abruptly or inexplicably during surgery. Among the alternative differential diagnoses – such as pulmonary emboli, obstruction or disconnection of the endotracheal tube – an unintended gastric perforation may be a life-threatening event, especially if unnoticed. After excluding other possible causes, close communication between the parties in the operating room could lead to the finding and the subsequent prompt repair of the perforated stomach. Finally, our findings would also be of clinical relevance with regard to patients with a perforated viscus who, as reported by Wiesel and co-authors [16], could present a palpable innocent abdomen and thus remain undiagnosed.

CONCLUSIONS

End-tidal carbon dioxide and VP commonly increase when pneumoperitoneum is applied. When the rat stomach was perforated, E_TCO_2 decreased and VP increased more than in the absence of perforation, these being more prominent under higher pressure conditions. The perforated stomach may allow for pressure-dependent CO_2 leakage and accelerated absorption by the gastric mucosa, both diminishing the detectable E_TCO_2 and increasing VP. We suggest that an abrupt or inexplicable decrease in E_TCO_2 or increase in VP during laparoscopic surgery, especially of the upper gastrointestinal tract, could be a warning sign of gastric perforation.

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Capsule

Phosphorylation of NLRC4 is critical for inflammasome activation

NLRC4 is a cytosolic member of the NOD-like receptor family that is expressed in innate immune cells. It senses indirectly bacterial flagellin and type III secretion systems, and responds by assembling an inflammasome complex that promotes caspase-1 activation and pyroptosis. Qu et al. used knock-in mice expressing NLRC4 with a carboxy-terminal 3×Flag tag to identify phosphorylation of NLRC4 on a single, evolutionarily conserved residue, Ser 533, following infection of macrophages with *Salmonella enterica* serovar Typhimurium (also known as *Salmonella typhimurium*). Western blotting with a NLRC4 phospho-Ser 533 antibody confirmed that this post-translational modification occurs only in the presence of stimuli known to engage NLRC4 and not the related protein NLRP3 or AIM2. *Nlrc*4−/− macrophages reconstituted with NLRC4 mutant S533A, unlike those reconstituted with wild-type NLRC4, did not activate caspase-1 and pyroptosis in response to *S. typhimurium*, indicating that S533 phosphorylation is critical for NLRC4 inflammasome function. Conversely, phosphomimetic NLRC4 S533D caused rapid macrophage pyroptosis without infection. Biochemical purification of the NLRC4-phosphorylating activity and a screen of kinase inhibitors identified PRKCD (PKCδ) as a candidate NLRC4 kinase. Recombinant PKCδ phosphorylated NLRC4 S533 in vitro, immunodepletion of PKCδ from macrophage lysates blocked NLRC4 S533 phosphorylation in vitro, and *Prkcd*−/− macrophages exhibited greatly attenuated caspase-1 activation and IL-1β secretion specifically in response to *S. typhimurium*. Phosphorylation-defective NLRC4 S533A failed to recruit procaspase-1 and did not assemble inflammasome specks during *S. typhimurium* infection, so phosphorylation of NLRC4 S533 probably drives conformational changes necessary for NLRC4 inflammasome activity and host innate immunity.

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Capsule

Structure of the agonist-bound neurotensin receptor

Neurotensin (NTS) is a 13-amino acid peptide that functions as both a neurotransmitter and a hormone through the activation of the neurotensin receptor NTSR1, a G protein-coupled receptor (GPCR). In the brain, NTS modulates the activity of dopaminergic systems, opioid-independent analgesia, and the inhibition of food intake; in the gut, NTS regulates a range of digestive processes. White et al. present the structure at 2.8Å resolution of *Rattus norvegicus* NTSR1 in an active-like state, bound to NTS$_{8-13}$, the carboxy terminal portion of NTS responsible for agonist-induced activation of the receptor. The peptide agonist binds to NTSR1 in an extended conformation nearly perpendicular to the membrane plane, with the C terminus oriented towards the receptor core. These findings provide, to our knowledge, the first insight into the binding mode of a peptide agonist to a GPCR and may support the development of non-peptide ligands that could be useful in the treatment of neurological disorders, cancer and obesity.

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