



Carbon dioxide pneumoperitoneum prevents mortality from sepsis

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Abstract

Background: Carbon dioxide (CO₂) pneumoperitoneum has been shown to attenuate the inflammatory response after laparoscopy. This study tested the hypothesis that abdominal insufflation with CO₂ improves survival in an animal model of sepsis and investigated the associated mechanism.

Methods: The effect of CO₂, helium, and air pneumoperitoneum on mortality was studied by inducing sepsis in 143 rats via intravenous injection of lipopolysaccharide (LPS). To test the protective effect of CO₂ in the setting of a laparotomy, an additional 65 animals were subjected to CO₂ pneumoperitoneum, helium pneumoperitoneum, or the control condition after laparotomy and intraperitoneal LPS injection. The mechanism of CO₂ protection was investigated in another 84 animals. Statistical significance was determined via Kaplan–Meier analysis for survival and analysis of variance (ANOVA) for serum cytokines.

Results: Among rats with LPS-induced sepsis, CO₂ pneumoperitoneum increased survival to 78%, as compared with using helium pneumoperitoneum (52%; $p < 0.05$), air pneumoperitoneum (55%; $p = 0.09$), anesthesia control (50%; $p < 0.05$), and LPS-only control (42%; $p < 0.01$). Carbon dioxide insufflation also significantly increased survival over the control condition (85% vs 25%; $p < 0.05$) among laparotomized septic animals, whereas helium insufflation did not (65% survival). Carbon dioxide insufflation increased plasma interleukin-10 (IL-10) levels by 35% compared with helium pneumoperitoneum ($p < 0.05$), and by 34% compared with anesthesia control ($p < 0.05$) 90 min after LPS stimulation. Carbon dioxide pneumoperitoneum resulted in a threefold reduction in tumor necrosis factor- α (TNF- α) compared with helium pneumoper-

itoneum ($p < 0.05$), and a sixfold reduction with anesthesia control ($p < 0.001$).

Conclusion: Abdominal insufflation with CO₂, but not helium or air, significantly reduces mortality among animals with LPS-induced sepsis. Furthermore, CO₂ pneumoperitoneum rescues animals from abdominal sepsis after a laparotomy. Because IL-10 is known to downregulate TNF- α , the increase in IL-10 and the decrease in TNF- α found among the CO₂-insufflated animals in our study provide evidence for a mechanism whereby CO₂ pneumoperitoneum reduces mortality via IL-10-mediated downregulation of TNF- α .

Key words: Carbon dioxide — Laparoscopy — Pneumoperitoneum — Sepsis — Surgery — Survival

When laparoscopy was first introduced into general surgery in the late 1980s [22], it was limited to elective procedures performed in healthy patients. As surgeons have become more comfortable with the new technology, the applications of laparoscopic surgery have expanded to include longer, more complex operations. The indications for laparoscopy have now evolved to include the sickest patients, because laparoscopy is now recognized as an accurate method for diagnosing and potentially treating causes of abdominal sepsis experienced by intensive care unit (ICU) patients [11, 20, 23].

Work from our institution and others have shown that carbon dioxide (CO₂) pneumoperitoneum attenuates the inflammatory response after laparoscopy. These data include basic scientific evidence that insufflation with CO₂ blunts the hepatic expression of acute phase genes in multiple models of perioperative sepsis [1, 14, 16], and clinical evidence that the release of inflammatory mediators is less after laparoscopy than after conventional open surgery [18, 27]. These data are consistent with the findings that patients subjected to laparoscopy generally experience less postoperative pain, shorter postoperative ileus, shorter hospital stays, and a more rapid return to preoperative activity than

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their laparotomized counterparts [4, 6, 12, 19]. Furthermore, the data clearly support the notion that CO₂ pneumoperitoneum has a specific biologic effect on the inflammatory response. However, the acute beneficial effects of CO₂ pneumoperitoneum have not yet been shown to correlate directly with the ultimate clinical outcome—an improvement in survival.

High serum levels of tumor necrosis factor- α (TNF- α) and other proinflammatory cytokines have been shown to correlate with mortality among patients with sepsis [7, 13], and the antiinflammatory cytokine interleukin-10 (IL-10) has been shown to be protective in this context [17, 25]. Furthermore, IL-10 has been shown to down-regulate TNF- α in multiple surgical models of sepsis [8, 21, 24]. Therefore, we hypothesized (a) that abdominal insufflation with CO₂ would improve survival from endotoxic shock, (b) that serum levels of TNF- α would correlate with mortality in this model and thus be lower in animals insufflated with CO₂, and (c) that decreased TNF- α levels in animals that have undergone CO₂ pneumoperitoneum would correlate with increased serum levels of IL-10.

Material and methods

General procedures

All procedures were part of an animal protocol reviewed and approved by the Johns Hopkins Medical Institutions Animal Care and Use Committee. Male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA) 10 to 12 weeks old and weighing between 250 and 300 g were housed in cages with standard chow and water available *ad libitum*. The animal housing environment was maintained at a temperature of 22°C with a 12-h light/dark cycle. The rats were acclimated to their environment for 3 to 5 days upon arrival and then fasted overnight before intervention. All procedures were performed under aseptic conditions. Anesthesia was induced in an isoflurane chamber for all the animals.

Maintenance vaporized isoflurane was delivered through a nose cone to the animals in experiments 1 and 2. The animals in experiment 3 were maintained in anesthesia with pentobarbital (50 mg/kg, intraperitoneal injection). Lipopolysaccharide (LPS) was from *Escherichia coli* serotype 026:B6 (Sigma-Aldrich, St. Louis, MO, USA). Pneumoperitoneum was achieved by delivering each respective gas through an 18-gauge angiocatheter placed percutaneously through the abdominal wall. Insufflation pressure was maintained at 3 to 4 mmHg. For survival studies, the animals were given *ad libitum* access to standard chow and water postprocedurally.

Effect of pneumoperitoneum on survival from sepsis

A total of 143 rats were anesthetized and then randomized into the following groups: CO₂ pneumoperitoneum, air pneumoperitoneum, helium pneumoperitoneum, anesthesia control, and LPS-only control. Animals assigned to the first four groups then received their respective pneumoperitoneum or anesthesia control treatment for 30 min, followed by a 120-min recovery period. All the animals then were injected with the previously determined median lethal dose (LD₅₀) of LPS (8 mg/kg, intravenous via the penile vein). The animals were observed continuously for 36 h after LPS injection, during which time mortality was documented.

Pneumoperitoneum protection in the setting of a laparotomy

A total of 65 rats were anesthetized and then randomized into the following groups: CO₂ pneumoperitoneum, helium pneumoperitoneum,

anesthesia control, laparotomy control, and LPS-only control. All the animals except those in the final control group then underwent a 5-cm midline laparotomy and received the LD₅₀ of LPS (8 mg/kg, intraperitoneal in the right colic gutter). The animals assigned to the LPS-only control group received their LPS via intraperitoneal injection through the abdominal wall. All laparotomies were immediately repaired using a double-layer 4-0 Vicryl closure. Each animal in the first three groups then received its respective treatment for 30 min. Animals in the laparotomy control group were allowed to recover from anesthesia immediately after abdominal closure. The animals were observed continuously for 36 h after LPS administration, during which time mortality was documented.

Mechanism of protection

A total of 84 rats were anesthetized and injected with a stimulatory dose of LPS (1 mg/kg, intravenous via the penile vein). The animals then were randomized into the following groups: anesthesia control, CO₂ pneumoperitoneum, and helium pneumoperitoneum. Each animal received its respective treatment for 90 min, after which blood was harvested via cardiac puncture. Plasma was isolated via centrifugation and stored at -80°C. Plasma levels of TNF- α , and IL-10 protein were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Biosource, Camarillo, CA, USA). Additional control animals received saline instead of LPS.

Statistical analysis

Statistical significance for survival studies was determined via Kaplan–Meier analysis using the log-rank test for general significance and the Holm–Sidak method for multiple pairwise comparisons. Cytokine data are expressed as mean \pm standard error of the mean (SEM). The one-way analysis of variance (ANOVA) test was used to detect general differences in serum cytokine levels among all the groups. To elucidate specific significances in these parameters between groups, multiple pairwise comparisons were performed using Tukey's test. Differences between groups were considered significant when *p* values were less than 0.05. Analysis was performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and SigmaStat (SPSS Incorporated, Chicago, IL, USA) software.

Results

Clinical effectiveness of the LPS model

All the animals in all three experiments exhibited signs and symptoms consistent with endotoxemic sepsis (piloerection, trembling, hypoactivity, diarrhea, decreased feeding, and conjunctival injection). The animals that received the LD₅₀ of LPS (experiments 1 and 2) began dying within 2 h of LPS injection. None of the animals that received the stimulatory dose of LPS (experiment 3) died before the blood harvest.

Survival benefit from insufflation with CO₂

To determine the effect of CO₂ pneumoperitoneum on survival from LPS-induced sepsis, rats were treated with CO₂ (or air or helium) abdominal insufflation before endotoxin injection (Fig. 1). Without treatment, 36-h survival from endotoxic sepsis was 42%. However, treatment with CO₂ pneumoperitoneum increased survival to 78% (*p* < 0.01). Treatment with helium pneumoperitoneum (survival, 52%), air pneumoperitoneum (survival, 55%), and anesthesia alone (survival, 50%) did

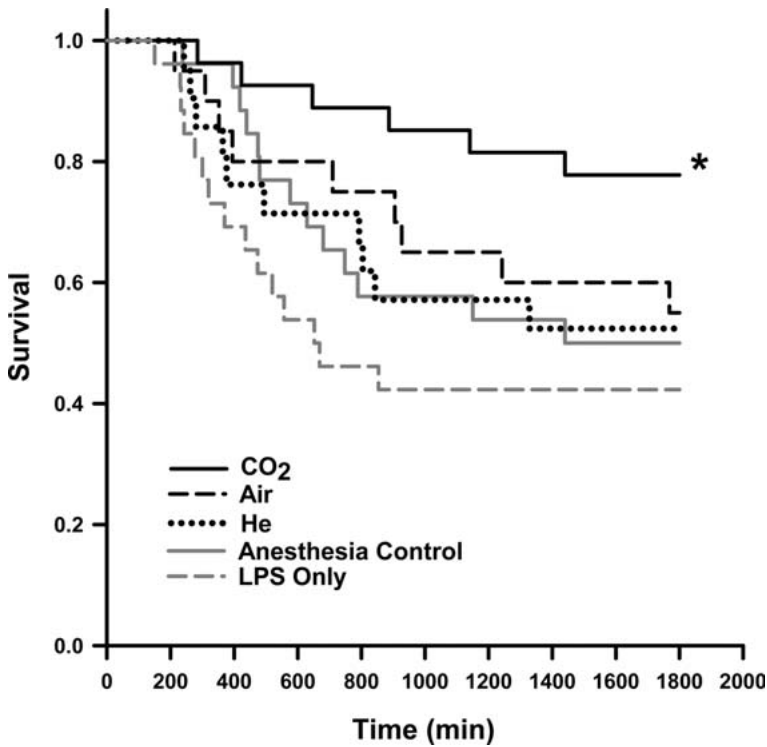


Fig. 1. Kaplan–Meier survival analysis among rats ($n = 143$) treated for 30 min with carbon dioxide pneumoperitoneum (CO₂), air pneumoperitoneum (Air), helium pneumoperitoneum (He), anesthesia only (Anesthesia Control), or nothing (LPS only) 2 h before a lethal dose of lipopolysaccharide (LPS, 8 mg/kg, intravenous). *CO₂ pneumoperitoneum increased survival to 78% compared with helium pneumoperitoneum (52%; $p < 0.05$), air pneumoperitoneum (55%; $p = 0.09$), anesthesia control (50%; $p < 0.05$), and LPS only control (42%; $p < 0.01$).

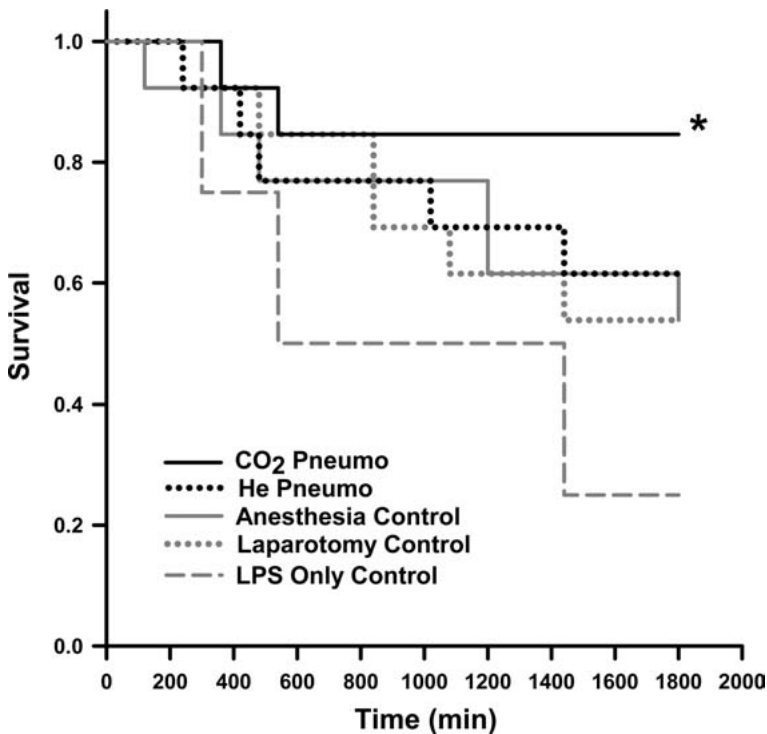


Fig. 2. Kaplan–Meier survival analysis among laparotomized rats ($n = 65$) treated for 30 min with carbon dioxide pneumoperitoneum (CO₂), helium pneumoperitoneum (He), anesthesia only (Anesthesia Control), or nothing (Laparotomy Control) immediately after a lethal dose of lipopolysaccharide (LPS, 8 mg/kg, intraperitoneal). The LPS only control animals did not receive a laparotomy. *CO₂ insufflation significantly increased survival over that of the LPS only control group (85% vs 25%; $p < 0.05$), whereas helium insufflation did not (65% survival).

not increase survival significantly over the LPS control ($p > 0.2$ for all). The survival benefit afforded by insufflation with CO₂ also was significant, as compared with helium insufflation ($p < 0.05$) and anesthesia alone ($p < 0.05$). A trend toward increased survival after CO₂ pneumoperitoneum, as compared with air pneumoperitoneum, was observed, but this difference did not reach statistical significance ($p = 0.09$).

CO₂ rescues animals from sepsis after a laparotomy

To explore whether CO₂ insufflation also might protect animals from death attributable to sepsis associated with a laparotomy, rats were treated with CO₂ (or helium) abdominal insufflation immediately after laparotomy and intraperitoneal administration of endotoxin (Fig. 2). Without treatment, intraperitoneal injection of

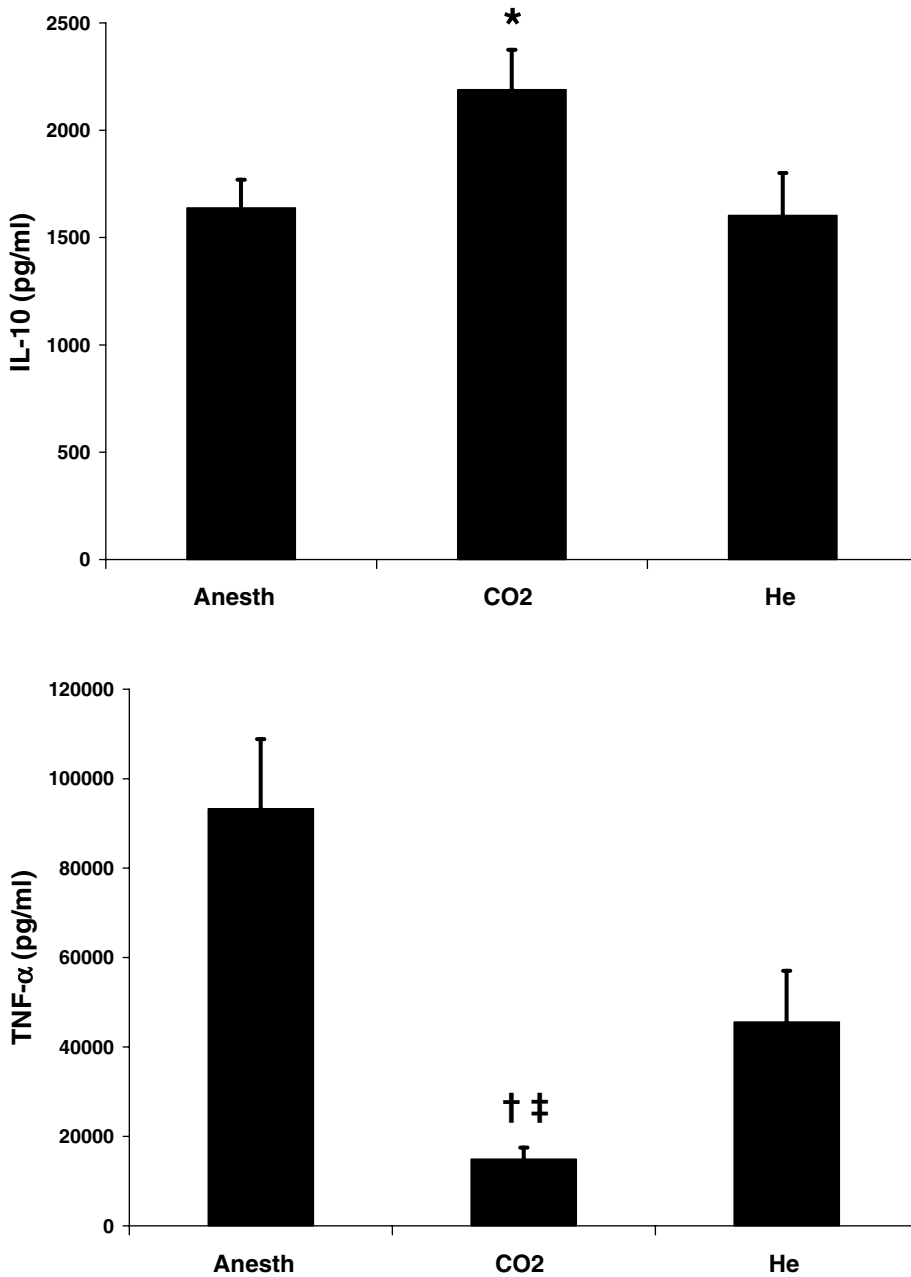


Fig. 3. Serum cytokine levels 90 min after lipopolysaccharide (LPS) injection among 84 rats treated with anesthesia alone (Anesth), carbon dioxide pneumoperitoneum (CO₂), or helium pneumoperitoneum (He). Insufflation with CO₂ increased plasma interleukin-10 (IL-10) levels by 35% compared with helium pneumoperitoneum, and by 34% compared with anesthesia control. Carbon dioxide pneumoperitoneum resulted in a threefold reduction in tumor necrosis factor- α (TNF- α) compared with helium pneumoperitoneum, and a sixfold reduction compared with the anesthesia control condition. Error bars represent \pm standard error of the mean (SEM). * $p < 0.01$ vs Anesth and He, † $p < 0.05$ vs He, ‡ $p < 0.001$ vs Anesth.

LPS resulted in only 25% survival. Treatment with CO₂ pneumoperitoneum significantly increased survival to 85% ($p < 0.05$), whereas treatment with helium pneumoperitoneum did not (survival, 65%; $p > 0.2$). Rats in the anesthesia control and laparotomy control groups also had intermediary survival (54% for both), but this survival was not significantly different from that associated with either LPS-only ($p > 0.2$ for both) or CO₂ pneumoperitoneum ($p > 0.1$ for both).

Humoral mechanism of CO₂ pneumoperitoneum protection

To investigate the humoral mechanism underlying the advantages of CO₂ insufflation observed in the aforementioned experiments, rats were treated with CO₂ or

helium pneumoperitoneum after stimulation with LPS. Insufflation with CO₂ increased plasma IL-10 levels by 35% compared with helium pneumoperitoneum ($p < 0.05$), and by 34% compared with anesthesia control ($p < 0.05$) 90 min after LPS administration (Fig. 3). Carbon dioxide pneumoperitoneum also resulted in a threefold reduction in serum TNF- α compared with helium pneumoperitoneum ($p < 0.05$), and a sixfold reduction compared with anesthesia control ($p < 0.001$). Stimulation with saline instead of LPS yielded levels of IL-10 and TNF- α that were virtually undetectable (data not shown).

Discussion

Because laparoscopy is known to attenuate humoral aspects of the inflammatory response [3, 18, 27], and

because lower serum levels of proinflammatory cytokines are known to correlate with lower mortality [7, 13], we investigated the effects of laparoscopy (focusing specifically on the effect of insufflation with CO₂) on survival among rats with lethal endotoxemia. We found that abdominal insufflation with CO₂, but not helium or air, significantly reduced mortality among animals with LPS-induced sepsis. Furthermore, we demonstrated that CO₂ pneumoperitoneum can even “rescue” from abdominal sepsis animals that already have been subjected to a laparotomy. The advantage afforded by CO₂ insufflation in our study represents a near doubling in the survival rate after LPS administration, as compared with no treatment.

In an effort to delineate the mechanism underlying the CO₂-specific survival benefit afforded by laparoscopy, we also measured serum cytokines in a similar, but nonlethal, experiment. The methodology of this experiment was necessarily different to ensure animal survival long enough for it to reach a time point known to represent the range of IL-10 and TNF- α serum peaks after rat LPS administration (60–120 minutes, data not shown) [26]. We found that insufflation with CO₂ increased plasma IL-10 levels by one-third compared with helium pneumoperitoneum (and compared with anesthesia control). Furthermore, CO₂ pneumoperitoneum resulted in a threefold reduction of serum TNF- α compared with helium insufflation, and a sixfold reduction compared with anesthesia alone.

Endotoxemic animals subjected to insufflation with CO₂ enjoyed longer survival, lower levels of TNF- α , and higher levels of IL-10 than animals receiving helium insufflation (our inert gas control for the mechanical effects of pneumoperitoneum). This suggests that a specific biologic effect of the CO₂ gas is responsible for the underlying mechanism of protection against mortality from endotoxemia. Because anesthesia alone has been shown to decrease inflammation and increase survival among septic animals [2, 9, 10], our anesthesia control groups are crucial to the correct interpretation of our data. Compared with these anesthesia control groups, helium pneumoperitoneum produced significantly lower TNF- α levels, but yielded similar IL-10 levels and mortality. This suggests that two mechanisms are involved in laparoscopy-associated attenuation of the inflammatory response. The mechanical effects of abdominal insufflation—mediated through pressure and stretch during abdominal expansion—are common to insufflation with any gas, and reduced TNF- α levels to a degree in our study, but presumably by an amount insufficient to affect survival. The specific biologic effect of CO₂ pneumoperitoneum in our study resulted in a further significant reduction of TNF- α production that correlated with a significant increase in survival. The fact that IL-10 levels were increased only in the animals subjected to the biologic activity of CO₂ suggests that IL-10-mediated suppression of TNF- α may be fundamental to the mechanism of CO₂-insufflation-specific protection against mortality from lethal endotoxemia. This finding is consistent with literature attesting to the inhibition of macrophage-derived TNF- α [8, 21, 24] and suppression of

nuclear factor κ B activation [30] (responsible for the upregulation of many proinflammatory genes) by IL-10, and with studies showing that administration of recombinant IL-10 increases survival in septic animals [17, 25].

The current study demonstrates that insufflation with CO₂ produces a survival benefit for animals that experience endotoxemia development, and suggests that this benefit may be secondary to attenuation of TNF- α proliferation, possibly via mediation through IL-10 stimulation. However, we have not shown how CO₂ stimulates IL-10 production (and/or TNF- α inhibition, if through a different mechanism). Carbon dioxide is quickly absorbed by the peritoneum, and has the opportunity to affect cytokine production by altering the function of peritoneal macrophages and/or Kupffer cells (via “communication” through the portal system), possibly via local acidification of the peritoneal environment. We have recently shown that abdominal insufflation with CO₂ does cause local peritoneal acidosis without affecting systemic acid–base status in properly ventilated animals [14, 15]. Furthermore, it has been shown that murine peritoneal macrophages derived from peritoneal cavities insufflated with CO₂ release less TNF- α in response to *in vitro* LPS stimulation than cells derived from animals insufflated with helium or air [29].

Future work should attempt to mimic the acidifying effect of CO₂ insufflation with a nongaseous acid, and should investigate the effects of CO₂ pneumoperitoneum in animals whose peritoneal macrophages have been depleted. Regarding the TNF- α -attenuating effects of mechanical abdominal expansion observed in the helium-insufflated animals in our study, future work should concentrate on the role of the vagus nerve and the cholinergic pathway, because the literature now suggests that the neurologic and immune systems are integrated into the body’s response to inflammation and injury [5, 28].

Our study illustrates that the CO₂ used in laparoscopic surgery plays an important role in the benefits conferred by minimally invasive surgery. The use of laparoscopy in trauma and other acute settings is increasing. Thus, the protective effect of CO₂ insufflation may make laparoscopy the immunologically preferred approach to diagnosis and therapy for patients with abdominal trauma and other potential causes of sepsis. It is even conceivable that peritoneal CO₂ insufflation alone might actually benefit patients critically ill with conditions characterized by unchecked inflammation. More likely, the biologic interaction between CO₂ and the immune system involves a pathway that can be precisely targeted pharmacologically in septic patients. Our demonstration that the CO₂ pneumoperitoneum during laparoscopy prevents mortality in an animal model of sepsis suggests that additional investigation in this area is warranted.

In conclusion, we have shown that abdominal insufflation with CO₂, but not helium or air, significantly increases survival among animals with LPS-induced sepsis. Furthermore, the protective effect of CO₂ pneumoperitoneum is capable of “rescuing” from abdominal sepsis animals that have already undergone a laparotomy. Incremental decreases in LPS-stimulated TNF- α

production via insufflation with an inert gas (helium) and a biologically active gas (CO₂) suggest that the inflammatory response after CO₂ laparoscopy is affected by both a mechanical mechanism and a biologic mechanism. Because IL-10 is known to downregulate TNF- α , the gas-specific increase in IL-10 found among the animals in our study insufflated with CO₂ provides evidence for a mechanism whereby CO₂ pneumoperitoneum reduces mortality from endotoxemia via IL-10-mediated downregulation of TNF- α . Our findings support the use of laparoscopy for the diagnosis and surgical treatment of patients with endotoxemia and/or significant inflammation.

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