CO_2 Abdominal Insufflation Pretreatment Increases Survival After a Lipopolysaccharide-Contaminated Laparotomy

Joseph M. Fuentes, M.D., Eric J. Hanly, M.D., Alexander R. Aurora, M.D., Antonio De Maio, Ph.D., Samuel P. Shih, M.D., Michael R. Marohn, D.O., Mark A. Talamini, M.D.

Carbon dioxide (CO_2)-pneumoperitoneum is known to favorably modify the systemic immune response during laparoscopic surgery. The presented studies were designed to determine whether treating animals with CO_2 abdominal insufflation before undergoing a lipopolysaccharide (LPS)-contaminated laparotomy would serve as "shock prophylaxis" and thus improve survival and attenuate cytokine production. Rats were randomized into five groups: CO_2-pneumoperitoneum, helium-pneumoperitoneum, anesthesia control, laparotomy/LPS control, and LPS only control. Animals in the first four groups all received a laparotomy and a lethal dose of LPS. Immediately preceding their laparotomy, animals in the pneumoperitoneum groups received a 30-minute pretreatment of abdominal insufflation with either CO_2 or helium. The anesthesia control group received a 30-minute pretreatment of isoflurane. Animal mortality was then recorded during the ensuing 72 hours. Subsequently, a similar protocol was repeated for measurements of cytokines. CO_2-pneumoperitoneum increased survival at 48 hours compared with LPS control ($P < .05$), and decreased interleukin-6 plasma levels at 2 hours ($P < .05$). Abdominal insufflation with CO_2 before the performance of a laparotomy contaminated with endotoxin increases survival and attenuates interleukin-6. The beneficial immune-modulating effects of CO_2-pneumoperitoneum endure after abdominal insufflation. CO_2-pneumoperitoneum pretreatment may improve outcomes among patients undergoing gastrointestinal surgery who are at high risk for abdominal fecal contamination. (J GASTROINTEST SURG 2006;10:32–38)

KEY WORDS: Carbon dioxide, pneumoperitoneum, laparotomy, sepsis, survival

Although laparoscopic surgery was initially viewed with skepticism, it was eventually received with enthusiasm as a minimally invasive means for removal of the gall bladder. Today laparoscopic surgery is used to perform a variety of complex procedures and is an integral part of surgical resident training.[1] Its popularity is attributable to decreased postoperative pain, faster recovery, decreased tissue trauma, and a more rapid return to oral intake.[2–5] Since its introduction, many studies have been performed to delineate which gas should be used to create the visual intraoperative field. Carbon dioxide (CO_2) has generally been accepted as the best gas because of its biologic properties: colorless, stable, buffered in the blood, low risk of venous embolism, and nonflammable/nonexplosive.[6,7]

Creating a pneumoperitoneum with CO_2 is only recently being recognized as an added benefit of laparoscopic surgery. CO_2-pneumoperitoneum has the added independent benefit of modifying the inflammatory response to injury. Our laboratory has shown that CO_2-pneumoperitoneum has the ability to attenuate the acute phase response in sepsis induced by LPS and cecal-ligation-puncture.[8,9] Furthermore, we have demonstrated that CO_2-pneumoperitoneum decreases tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6 production while up-regulating the anti-inflammatory cytokine IL-10.[10–12] Consistent with our findings, others have shown that CO_2-pneumoperitoneum reduces levels of TNF-α mRNA, IL-6, IL-1 receptor antagonist, and soluble TNF receptor-1.[13–15] More important, our

Presented at the Forty-Sixth Annual Meeting of The Society for Surgery of the Alimentary Tract, Chicago, Illinois, May 14–18, 2005 (poster presentation).

From The Department of Surgery, The Johns Hopkins University School of Medicine, Baltimore, Maryland.

Supported by R01-GM062899-02, National Institutes of Health, Bethesda, Maryland.

Correspondence: Mark A. Talamini, M.D., Department of Surgery, University of California-San Diego, 200 W. Arbor Drive, #8400, San Diego, CA 92103-8400. e-mail addresses: talamini@ucsd.edu; jfuentel@jhmi.edu and ehanly@jhmi.edu

Published by Elsevier Inc.

1091-255X/06/$—see front matter
doi:10.1016/j.gassur.2005.07.031

laboratory has shown that CO_2-pneumoperitoneum increases survival in rodents challenged with endotoxin.[16] However, the mechanism underlying this phenomenon is only partially understood. Although smaller incisions in laparoscopic surgery have been advocated as the main factor in decreasing the inflammatory response,[14] our research highlights the active biologic state induced by CO_2 as integral to the mechanism behind the potentially beneficial immunomodulatory properties observed during laparoscopic surgery. We have shown that CO_2 insufflation given immediately after an LPS-contaminated laparotomy (6 cm) protects rodents from endotoxic shock.[16]

Intra-abdominal infection and sepsis continue to be a problem in surgical and critically ill patients.[17,18] Sepsis continues to have a high mortality (30%–70%) despite advanced technology and intensive therapy.[19] This study explores the possibility of an alternative means of preventing intra-abdominal sepsis. We hypothesized that CO_2-pneumoperitoneum pretreatment would also offer protection against sepsis after a lipopolysaccharide (LPS)-contaminated laparotomy. Specifically, here CO_2 insufflation was used as a prophylactic agent to prevent the development of endotoxic shock.

MATERIALS AND METHODS

General Procedures

Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing approximately 250 to 350 g were housed in plastic cages where standard chow and water were available ad libitum. Animals were acclimatized to their environment for 3 to 5 days after arrival and were then fasted for 16 hours before procedures. The animal housing environment maintained a 12-hour light/dark cycle, a temperature of 72°F, and a humidity ranging between 30% and 70%. All surgical procedures were performed under aseptic conditions. Before surgery, 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 and restrained with adhesive tape. Anesthesia was maintained by delivering 2.5% vaporized isoflurane through a nose cone. Pneumoperitoneum was achieved by delivering each respective gas through an 18-gauge angiocatheter placed percutaneously through the abdominal wall. Insufflation pressure was maintained at 4 mm Hg. LPS was from *Escherichia coli* serotype 026:B6 (Sigma-Aldrich, St. Louis, MO). After the completion of the experiments, all rats were euthanized by anesthetic overdose. All animal procedures were performed on a protocol approved by Johns Hopkins Medical Institution Animal Care and Use Committee.

Survival Study and Lipopolysaccharide Model

Sixty-five rats were randomized into five groups \ (n = 13): CO_2-pneumoperitoneum, helium-pneumoperitoneum, anesthesia control, laparotomy/LPS control, and LPS only control. Animals in the first four groups all received a 6-cm midline laparotomy that was followed by instilling a lethal dose of LPS (10 mg/kg) in the right colic gutter. All laparotomies were immediately repaired using a double-layer 4-0 Vicryl closure. The LPS only control group received an intraperitoneal injection of LPS at the same dose. Immediately preceding their laparotomy, animals in the pneumoperitoneum groups received a 30-minute pretreatment of abdominal insufflation (4 mm Hg) with either CO_2 or helium. The anesthesia control group received a 30-minute pretreatment of isoflurane. Animal mortality was then recorded during the ensuing 72 hours.

Cytokine Response Study

Sixty-two rats (n = 8) were randomized into four groups: CO_2 pneumoperitoneum, helium pneumoperitoneum, anesthesia control, and laparotomy/LPS only control. The first three groups received 30 minutes of preconditioning with their respective treatments followed by a 6-cm midline laparatomy for 10 to 30 minutes. Simultaneous to laparotomy, rats received intraperitoneal LPS (1 mg/kg) in the right colic gutter. The laparotomy/LPS only control group received no preconditioning. Blood samples for serum cytokine assays were collected through cardiac puncture 2 hours after LPS injection. Plasma was isolated by centrifugation and stored at −80°C. Plasma levels of TNF-α, IL-6, and IL-10 protein were determined by enzyme-linked immunosorbent assay using commercially available kits (Biosource, Camarillo, CA).

Statistical Analysis

Statistical significance for survival studies was determined by Kaplan-Meier analysis using the log-rank test. Cytokine data are expressed as mean ± standard error of the mean. One-way analysis of variance test was used to detect general differences in serum cytokine levels among all groups. To elucidate specific significances in these parameters between groups, multiple pairwise comparisons were performed using the Student-Newman-Kelus method.

Differences between groups were considered significant when the *P* value was less than .05. Analysis was performed using Microsoft Excel (Microsoft Corporation, Redmond, WA) and SigmaStat (SPSS Incorporated, Chicago, IL) software.

RESULTS

Survival Study

To determine whether CO_2 pneumoperitoneum pretreatment for 30 minutes had the potential to prevent the detrimental effects of an LPS-contaminated laparotomy, a survival study was performed. Figure 1 demonstrates that at 24 hours after an LPS-contaminated laparotomy was performed, there was no significant difference in mortality among groups ($P = 0.317$ for CO_2 vs. LPS control). However, CO_2-pneumoperitoneum significantly increased survival compared with LPS control at 48 hours (85% vs. 46%, $P = .037$). Helium pneumoperitoneum, laparotomy/LPS control, and anesthesia control had intermediary survival (77% vs. 46%, $P = .124$; 62% vs. 46%, $P = .387$; 69% vs. 46%, $P = .278$, respectively). At 72 hours, a trend

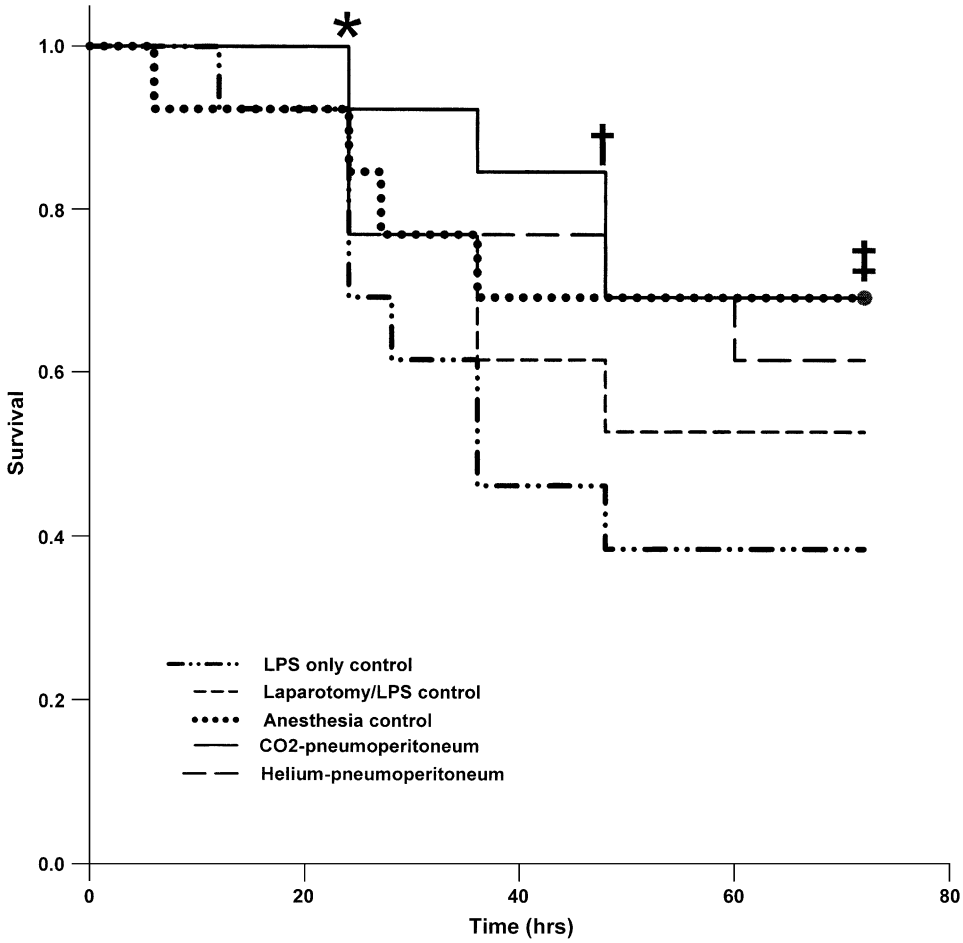

Fig. 1. Kaplan-Meier survival analysis among rats (n = 65) pretreated for 30 minutes with CO_2 pneumoperitoneum, helium pneumoperitoneum, or anesthesia control. Laparotomy/LPS control received no pretreatment, whereas LPS control animals did not receive a laparotomy. At 24 hours, there was no significant difference between groups, $^*P = .317$ for CO_2 versus LPS control. At 48 hours, CO_2-pneumoperitoneum significantly increased survival compared with LPS control (85% vs. 46%, $^\dagger P = .037$), whereas helium pneumoperitoneum, laparotomy/LPS control, and anesthesia had intermediary survival (77% vs. 46%, $P = .124$; 62% vs. 46%, $P = .387$; 69% vs. 46%, $P = .278$, respectively). Furthermore, at 72 hours CO_2-pneumoperitoneum increased survival compared with LPS control (69% vs. 38%, $^\ddagger P = .07$), whereas helium pneumoperitoneum, laparotomy/LPS control, and anesthesia had intermediary survival (62% vs. 38%, $P = .124$; 54% vs. 38%, $P = .400$; 69% vs. 38%, $P = .162$, respectively). CO_2 = Carbon dioxide; LPS = lipopolysaccharide.

existed toward CO_2 pneumoperitoneum increasing survival compared with LPS control, but this difference did not reach statistical significance (69% vs. 38%, $P = .07$), whereas helium pneumoperitoneum, laparotomy/LPS control, and anesthesia again had intermediary survival (62%vs. 38%, $P = .124$; 54% vs. 38%, $P = .400$; 69% vs. 38%, $P = 0.162$, respectively). Animals treated with CO_2-pneumoperitoneum exhibited fewer clinical signs of abdominal sepsis during the 72-hour observational period (e.g., piloerection, diarrhea, hypoactivity).

Cytokine Response Study

To determine whether our previous observations would correlate with a reduction in proinflammatory cytokine levels and an increase in the anti-inflammatory cytokine IL-10, a similar protocol was conducted. Plasma levels of the proinflammatory cytokine IL-6 were significantly suppressed in animals pretreated with CO_2-pneumoperitoneum compared with laparotomy control, anesthesia control, and helium pneumoperitoneum (71%, 66%, and 65%, respectively, $P < .05$, Fig. 2). Moreover, plasma levels of the proinflammatory cytokine TNF-α were suppressed in the group pretreated with CO_2 when compared with laparotomy control, anesthesia, and helium pneumoperitoneum (30%, 34%, and 28%, respectively), although this difference did not reach statistical significance (Fig. 3). IL-10 levels were not significantly different between groups, although there was a high IL-10 response among all groups.

DISCUSSION

The experiments presented herein were designed to determine whether pretreatment with CO_2-pneumoperitoneum for 30 minutes would serve as "shock prophylaxis" in animals whose peritoneal cavity was instilled with LPS during a laparotomy. We found that CO_2-pneumoperitoneum pretreatment prevented early mortality at 48 hours post-LPS injection in the setting of a laparotomy. Moreover, at 72 hours, CO_2 insufflation pretreated had borderline significance (statistical significance may been reached if our sample size was greater). This survival study corroborates the results of our prior work in which 30 minutes of CO_2-pnuemoperitoneum treatment increased survival even when the lethal dose of LPS was injected 2 hours after treatment, and in which CO_2 insufflation immediately after laparotomy and LPS administration increased 72-hour survival.[16] The current study confirms that CO_2 insufflation does indeed modify the inflammatory response induced by LPS, and most important, that

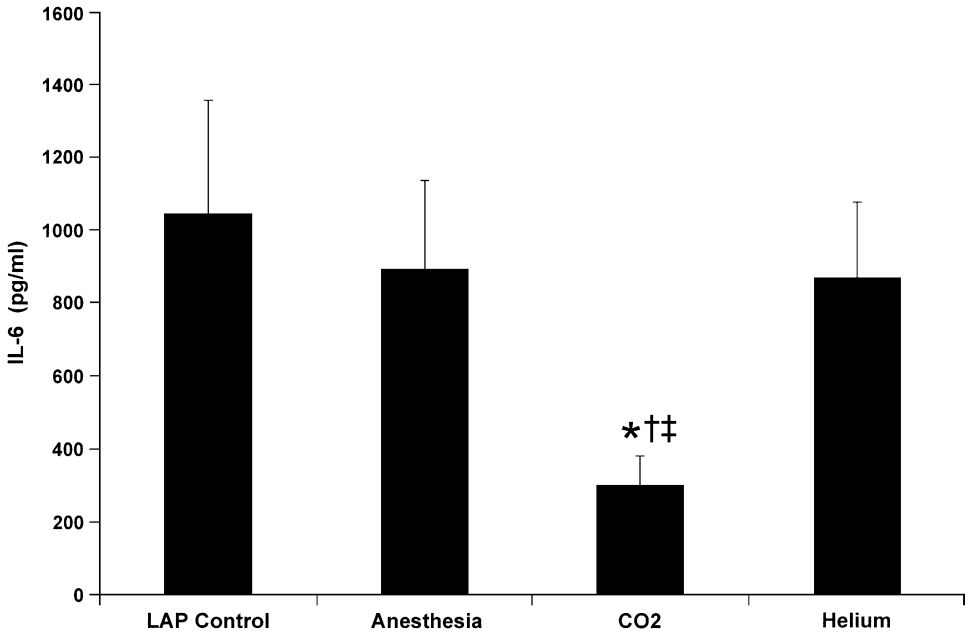

Fig. 2. Down-regulation of IL-6 plasma levels by 30 minutes pretreatment with CO_2 insufflation after a contaminated laparotomy. Plasma levels of IL-6 in animals pretreated with CO_2 pneumoperitoneum for 30 minutes were significantly suppressed when compared with laparotomy control, anesthesia control, and helium pneumoperitoneum (71%, 66%, and 65%, respectively). Data presented as mean ± SEM. *$P < .05$ versus LAP (laparotomy) control, †$P < .05$ versus anesthesia, ‡$P < .05$ versus helium pneumoperitoneum. IL = interleukin; SEM = Standard error of mean.

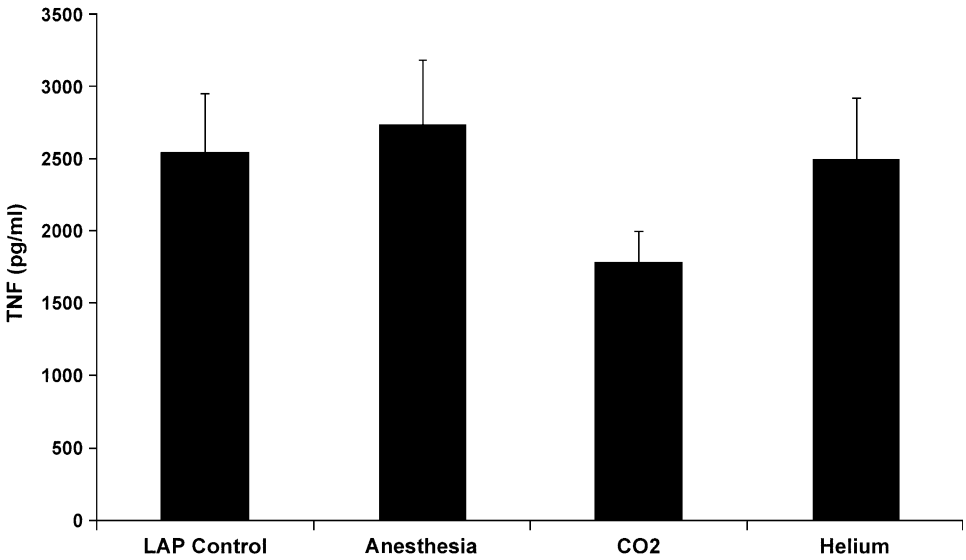

Fig. 3. TNF-α plasma levels after 30 minutes of their respective pretreatment after an LPS-contaminated laparotomy. Plasma levels of TNF-α in animals pretreated with CO_2 pneumoperitoneum were suppressed when compared with laparotomy control, anesthesia, and helium pneumoperitoneum (30%, 34%, and 28%, respectively), although these differences did not reach statistical significance by one-way analysis of variance. TNF = tumor necrosis factor; LAP = laparotomy; LPS, lipopolysaccharide.

the effects are durable, producing a prophylactic effect against subsequent laparotomy and intra-abdominal LPS administration. Furthermore, our cytokine data support the concept of a mechanism whereby CO_2 insufflation increases survival by attenuating the cytokine storm induced by LPS. We observed that IL-6 was significantly decreased in the CO_2 pneumoperitoneum group, whereas there was a trend toward TNF-α being suppressed. Consistent with these findings, we previously showed that CO_2-pneumoperitoneum decreases TNF-α and IL-6 responses sepsis.[11,12,16] Previous studies showed that cholecystectomy by laparoscopic surgery attenuates IL-6 whereas cholecystectomy by laparotomy has a significant elevation of IL-6.[14] Although the authors of the former study concluded that their results show that less surgical stress with laparoscopic surgery is the main cause of the difference between open laparotomy and laparoscopic surgery, in our study, animals pretreated with CO_2 insufflation and that then had a laparotomy still displayed decrease levels of IL-6. This suggests that the unique biology of the CO_2-pneumoperitoneum is more important than the size of incision.

The clinical implications of our study are that CO_2-pneumoperitoneum might have potential as a therapeutic agent to prevent or treat surgical sepsis. This study suggests that CO_2-pneumoperitoneum pretreatment may play a role as an adjuvant or a perioperative agent used in operations with a high risk of abdominal fecal contamination. CO_2 anti-inflammatory effects are not unique to minimally invasive surgery, but may be relevant in different clinical areas. CO_2 insufflation used in flexible endoscopy has gained increased popularity over air because of less pain and discomfort after the procedures.[20] It has been proposed that rapid CO_2 absorption is the main factor for less patient discomfort.[20] CO_2 insufflation is also applied to prevent airembolism, during drainage of subdural hematomas and during cardiac surgery.[21–23] Furthermore during cardiac surgery, CO_2 insufflation has also been reported to significantly reduce the risk of airborne contamination and postoperative wound infection.[24] All these benefits are attributed to the active biologic state of CO_2. This study provides evidence that other procedures in which CO_2 could be applied instead of air or saline to create a visual field should consider switching to CO_2.

One limitation of our study is that, in an attempt to measure cytokines levels at a time that would allow consideration of both TNF-α and IL-6, we harvested blood samples at 2 hours, when TNF-α and IL-10 generally peak somewhat earlier. In this study, plasma levels of TNF-α were modestly suppressed in the CO_2 group, and IL-10 levels were similar among the study groups. Only IL-6 was significantly suppressed in the CO_2 group when compared with the

other groups. At 2 hours, TNF-α and IL-10 levels already began to fall, making differences harder to appreciate between groups. This limitation in experimental design might explain why, in this study, IL-10 levels were not significantly up-regulated in the CO_2-pneumoperitoneum group as seen in our previous studies.[16] In this study, the laparotomy is known to cause an acute phase response with the release of proinflammatory cytokines such as TNF-α and IL-6 with the subsequent release of anti-inflammatory mediators such as IL-10 and transforming growth factor-beta.[25,26] Moreover, studies suggest that during a laparotomy the T-helper (Th1)/Th2 ratio shifts toward a Th2-dominated cytokine profile (anti-inflammatory), whereas during laparoscopic surgery the Th1/Th2 balance is maintained.[25,26] This may in part also explain the difference in cytokine profile in this study.

We showed previously that abdominal insufflation with CO_2 causes local peritoneal acidosis without affecting systemic acid-base status if properly ventilated.[27] West et al.[28] also showed that the cystosolic pH of macrophages significantly decreased with CO_2, whereas macrophages incubated with air and helium had no effect. Furthermore, West et al.[29] also showed that cultured peritoneal macrophages stimulated with LPS had a significant reversible decrease in TNF-α and IL-1 production when exposed to CO_2 in vitro with no inhibition with helium or air. This group suggests that local tissue acidification caused by peritoneal CO_2 insufflation might explain the decrease in the inflammatory response. Likewise, in previous studies we demonstrated that helium pneumoperitoneum does not cause local peritoneal acidosis or increase survival, whereas CO_2-pneumoperitoneum does cause local peritoneal acidosis and does increase survival in an LPS model of sepsis.[16,27] Taken together, CO_2-pneumoperitoneum–mediated acidification of the peritoneal cavity may explain the increase in survival. In this study, pretreatment with CO_2 insufflation "primed" the peritoneal cavity and prevented subsequent sequelae of endotoxic shock from laparotomy and a lethal dose of LPS.

Future work should be conducted to evaluate CO_2-pneumoperitoneum pretreatment in the face of a more clinically relevant model of sepsis such as cecal ligation and puncture. Furthermore, studies should be conducted to evaluate whether CO_2 protection observed in the abdominal cavity holds true in other macrophage-rich anatomic locations such as the pleural cavity. This study suggests that CO_2-pneumoperitoneum pretreatment may play an important role as an adjuvant or perioperative agent used in operations with a high risk of abdominal fecal contamination. Studies should also attempt to elucidate the specific mechanism whereby CO_2-pneumoperitoneum—whether by acidification or other means—"primes" the peritoneal cavity to subsequent infection or injury.

CONCLUSION

Our data demonstrate that 30 minutes of CO_2-pneumoperitoneum pretreatment increases survival among animals subjected to subsequent LPS-contaminated laparotomy. Furthermore, CO_2-pneumoperitoneum pretreatment significantly decreases IL-6 plasma levels 2 hours after LPS-contaminated laparotomy. Finally, this study further supports our and others' findings that CO_2-pneumoperitoneum is a potent anti-inflammatory agent with possible therapeutic properties.

REFERENCES

1. Feldman LS, Hagarty SE, Ghitulescu G, Stanbridge D, Fried GM. Relationship between objective assessment of technical skills and subjective in-training 12 residents. J Am Coll Surg 2004;198:105–110.
2. Barkun JS, Wexler MJ, Hinchey EJ, Thibeault D, Meakins JL. Laparoscopic versus open inguinal herniorrhaphy: preliminary results of a randomized controlled trial. Surgery 1995;118:703–709.
3. Jatzko GR, Lisborg PH, Pertl AM, Stettner HM. Multivariate comparison of complications after laparoscopic cholecystectomy and open cholecystectomy. Ann Surg 1995;221:381–386.
4. Buanes T, Mjaland O. Complications in laparoscopic and open cholecystectomy: a prospective comparative trial. Surg Laparosc Endosc 1996;6:266–272.
5. Glaser F, Sannwald GA, Buhr HJ, et al. General stress response to conventional and laparoscopic cholecystectomy. Ann Surg 1995;221:372–380.
6. Mann C, Boccara G, Grevy V, Navarro F, Fabre JM, Colson P. Argon pneumoperitoneum is more dangerous than CO2 pneumoperitoneum during venous gas embolism. Anesth Analg 1997;85:1367–1371.
7. Sietses C, von Blomberg ME, Eijsbouts QA, Beelen RH, Berends FJ, Cuesta MA. The influence of CO2 versus helium insufflation or the abdominal wall lifting technique on the systemic immune response. Surg Endosc 2002;16:525–528.
8. Are C, Talamini MA, Murata K, De Maio A. Carbon dioxide pneumoperitoneum alters acute-phase response induced by lipopolysaccharide. Surg Endosc 2002;16:1464–1467.
9. Hanly EJ, Mendoza-Sagaon M, Murata K, Hardacre JM, De Maio A, Talamini MA. CO_2 pneumoperitoneum modifies the inflammatory response to sepsis. Ann Surg 2003;237:343–350.
10. Hanly EJ, Bachman SL, Marohn MR, et al. CO_2-pneumoperitoneum-mediated attenuation of the inflammatory response is independent of systemic acidosis. Surgery 2005;137:559–566.
11. Fuentes JM, Hanly EJ, Aurora AR, De Maio A, Marohn MR, Talamini MA. Carbon dioxide pneumoperitoneum posttreatment attenuates IL-6 production. Surg Endosc 2005;19:S226.

12. Aurora AR, Fuentes JM, Hanly EJ, De Maio A, Talamini MA. CO_2 pneumoperitoneum attenuates IL-6 production in peritoneal macrophages. J Surg Res 2004;121:336.
13. Kamei H, Yoshida S, Yamasaki K, Tajiri T, Shirouzu K. Carbon dioxide pneumoperitoneum reduces levels of TNF-α mRNA in the brain, liver, and peritoneum in mice. Surg Endosc 2001;15:609–613.
14. Schietroma M, Carlei F, Franchi L, et al. A comparison of serum interleukin-6 concentrations in patients treated by cholecystectomy via laparotomy or laparoscopy. Hepatogastroenterology 2004;51:1595–1599.
15. Yoshida S, Ohta J, Yamasaki K, et al. Effect of surgical stress on endogenous morphine and cytokine levels in the plasma after laparoscopoic or open cholecystectomy. Surg Endosc 2000;14:137–140.
16. Hanly EJ, Fuentes JM, Aurora AR, et al. CO_2 pneumoperitoneum prevents mortality from sepsis. Surg Endosc 2005; 19:S176.
17. Paterson DL, Rossi F, Baquero F, et al. In vitro susceptibilities of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: the 2003 Study for Monitoring Antimicrobial Resistance Trends (SMART). J Antimicrob Chemother 2005;55:965–973.
18. Marshall JC. Intra-abdominal infections. Microbes Infect 2004;6:1015–1025.
19. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003;348:138–150.
20. Bretthauer M, Hoff G, Thiis-Evensen E, et al. Carbon dioxide insufflation reduces discomfort due to flexible sigmoidoscopy in colorectal cancer screening. Scand J Gastroenterol 2002;37:1103–1107.
21. Kubo S, Takimoto H, Nakata H, Yoshimine T. Carbon dioxide insufflation for chronic subdural haematoma: a simple addition to burr-hole irrigation and closed-system drainage. Br J Neurosurg 2003;17:547–550.
22. Martens S, Theisen A, Balzer JO, et al. Improved cerebral protection through replacement of residual intracavital air by carbon dioxide: a porcine model using diffusion-weighted magnetic resonance imaging. J Thorac Cardiovasc Surg 2004;127:51–60.
23. Nichols HT, Morse DP, Hirose T. Coronary and other air embolization occurring during open cardiac surgery; prevention by the use of gaseous carbon dioxide. Surgery 1958;43: 236–244.
24. Persson M, van der Linden J. Wound ventilation with carbon dioxide: a simple method to prevent direct airborne contamination during cardiac surgery? J Hosp Infect 2004;56: 131–136.
25. Brune IB, Wilke W, Hensler T, Holzmann B, Siewert JR. Downregulation of T helper type 1 immune response and altered pro-inflammatory and anti-inflammatory T cell cytokine balance following conventional but not laparoscopic surgery. Am J Surg 1999;177:55–60.
26. Menger MD, Vollmar B. Surgical trauma: hyperinflammation versus immunosuppression? Langenbecks Arch Surg 2004;389:475–484.
27. Hanly EJ, Fuentes JM, Aurora AR, et al. Gastroenterology 2005;128:A783.
28. West MA, Hackam DJ, Baker J, Rodriguez JL, Bellingham J, Rotstein OD. Mechanism of decreased in vitro murine macrophage cytokine release after exposure to carbon dioxide: relevance to laparoscopic surgery. Ann Surg 1997;226:179–190.
29. West MA, Baker J, Bellingham J. Kinetics of decreased LPS-stimuIated cytokine release by macrophages exposed to CO_2. J Surg Res 1996;63:269–274.