The Effect of Warm Humidified CO_2 on the Dissipation of Residual Gas Following Laparoscopy in Piglets

Peter A. Glew, M.B., B.Ch., Matthys J. J. Campher, M.B., Ch.B., Kay Pearson, NZAScT, John C. Schofield, B.VSc., and Andrew K. Davey, Ph.D, M.P.S.

Abstract *(J Am Assoc Gynecol Laparosc 2004, 11(2):204–210)*

Study Objective. *To determine whether residual gas volume reduces more quickly after insufflation with humidified CO_2 compared with dry CO_2.*

Design. *Animal study (Canadian Task Force classification I).*

Setting. *University.*

Intervention. *Piglets were randomly divided into two groups of five and underwent abdominal insufflation with either cold, dry CO_2 or warm, humidified CO_2.*

Measurements and Main Results. *Following insufflation, anteroposterior and lateral gas-bubble radiographic images were obtained at 5, 15, 30, 45, and 60 minutes, and the area of each gas-bubble profile calculated. Blood samples were obtained at 0, 2, 4, and 5 hours, and they were analyzed for IL-1β and TNFα. Peritoneal tissue samples were obtained on euthanasia at 5 hours for histological analysis. The results indicate that following pneumoperitoneum, residual CO_2 dissipates more rapidly when the gas is heated and humidified compared with when it is cool and dry. This is associated with a reduction in the duration of the inflammatory response as measured by TNFα production, although no histologic differences in the peritoneal tissue were observed.*

Conclusion. *Heating and humidifying CO_2 leads to faster dissipation of residual gas associated with a reduced duration of inflammation, which may contribute toward a reduction in postlaparoscopic pain.*

Although laparoscopic surgery offers many advantages compared with conventional surgery, a number of improvements can still be made. After laparoscopic surgery, postoperative pain and discomfort are still present to a significant extent. The need to insufflate the abdomen with gas to allow space for operational maneuvers leading to distention of abdominal tissue and changes within the peritoneal lining is thought to be the cause of most of the postoperative pain.[1]

Currently, insufflation is achieved using cold, dry CO_2. Evaporation of water from the abdominal cavity into dry gas can cause a number of effects, including desiccation of the tissue leading to peritoneal cell injury and death, an inflammatory response, and subsequent adhesion formation.[1–3] The type and condition of gas used has a significant influence on the inflammation, as measured by the cytokine response.[4–7]

One method employed to counteract some of the effects of the insufflating gas is to pre-humidify the gas. This prevents evaporation of water from the peritoneum, reducing the likelihood of hypothermia,[8,9] and may reduce tissue damage caused by dehydration and the resulting inflammatory processes.[6,10] It is thought that this reduction in tissue damage and inflammation is one reason why humidified gas can lead to reduced postoperative pain, shorter hospital stays, and a better clinical outcome.[1,2,11] Furthermore, there is a significant correlation between postlaparoscopy pain, particularly right shoulder tip pain, and the size of the residual gas bubble (both the volume and the arc) under the right hemidiaphragm.[12] It is therefore not surprising that the removal of residual CO_2, particularly from the subdiaphragmatic region, leads to a reduction in pain.[13,14] The prevention of dehydration of the peritoneal lining by prehumidification may have an important effect, as this should maintain a moist surface that, in theory, allows CO_2 to dissolve and so diffuse more rapidly away from the peritoneum. If this is the case, then the use of humidified CO_2 could lead to a more rapid removal of residual gas bubbles with a subsequent reduction and/or shorter duration of pain.

From the Department of Anesthesia (Drs. Glew and Campher), the Department of Orthopaedics (Mrs. Pearson), The Animal Welfare Office (Dr. Schofield), University of Otago, Dunedin, New Zealand; and the School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia (Dr. Davey).

Corresponding author Andrew K. Davey, Ph.D., M.P.S., University of South Australia, School of Pharmacy and Medical Sciences, Division of Health Sciences, City East Campus, GPO Box 2471, Adelaide SA 5001, Australia.

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The primary aim of this study was to determine whether the residual gas bubble reduces more quickly after insufflation with humidified CO_2 compared with dry CO_2. As secondary aims, cell histology and serum cytokine concentrations were determined to give an indication of any effects of CO_2 humidification on the peritoneal tissue damage and inflammatory response.

Methods

Ten piglets (large white breed), 5- to 7-days old (2.5 to 3.5 kg), were randomly divided into two groups of five and underwent abdominal insufflation with either dry CO_2 or humidified CO_2, as described below. The randomization was determined by the order of arrival of the animals in the housing facility, with alternate animals being assigned to control or treatment groups, respectively. All procedures were approved by the University of Otago Animal Ethics Committee.

Experimental Procedure

The piglets were held in a customized pen under a heat lamp on sawdust bedding and fasted overnight. Each animal was premedicated with xylazine 1 mg/kg and ketamine 5 mg/kg given intramuscularly in the hind leg. Once sedated, anesthesia was induced with 3%–4% halothane in oxygen delivered by a nose cone using a Mapleson F circuit and Jackson Rees bag. The vocal cords were sprayed with 5 mg of lidocaine prior to intubation with 3.0 cuffed endotracheal tube. An oral gastric tube was inserted to decompress the stomach. Anesthesia was maintained throughout the procedure with 0.8%–1.5% halothane and oxygen by spontaneous breathing or by ventilation using a Nuffield ventilator (Penlon, Abingdon, UK) with a Newton valve.

Each piglet was placed into a customized cradle and secured in dorsal recumbency. Body temperature was maintained by a whole-body insulating wrap and monitored at regular intervals. The jugular vein was surgically exposed and cannulated with a 22-gauge nonported catheter. A 0.9% normal saline cannula lock was used. Animals were monitored by pulse oximetry and end-tidal capnography. A right iliac fossa skin incision was made for the insertion of two 12-cm long Veress needles, with the tips placed within the peritoneal cavity in the left flank and right subcostal regions. The needles were secured in place, and an air-tight seal was maintained using sutures.

A pilot humidification system (Fisher & Paykel Healthcare, Auckland, New Zealand) was used to deliver heated and humidified CO_2 gas (Figures 1, 2). The connection tube shown in Figure 2 attaches to the insufflator. The cold, dry gas from the insufflator flows through the connection tube and passes through a filter into a humidification chamber. The humidification chamber contains sufficient water to prevent the need for refilling during a surgical procedure. The water is heated by a heater plate in the heater base, and the vapor above the water mixes with the cold gas so the gas exiting the chamber is at body temperature and saturated with water vapor (37° C, 100% relative humidity

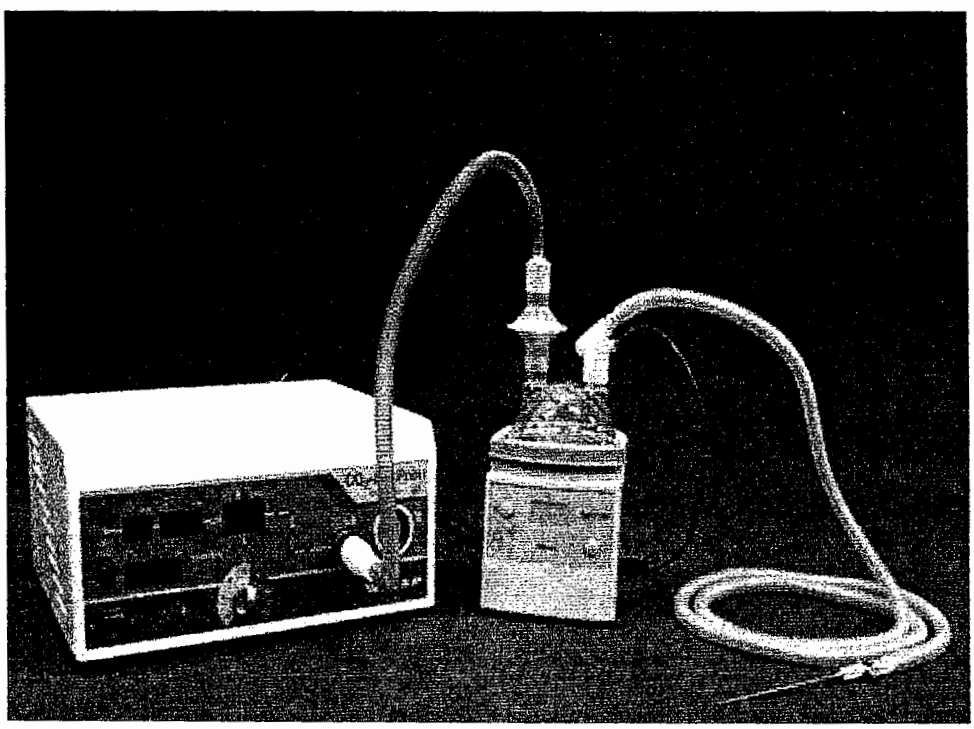

FIGURE 1. Study equipment: Insufflator, humidification system (consisting of a connection tube, filter, humidification chamber and heated insulated tube, electrical adapter with flow and temperature sensors, and heater base controller), and Veress needle.

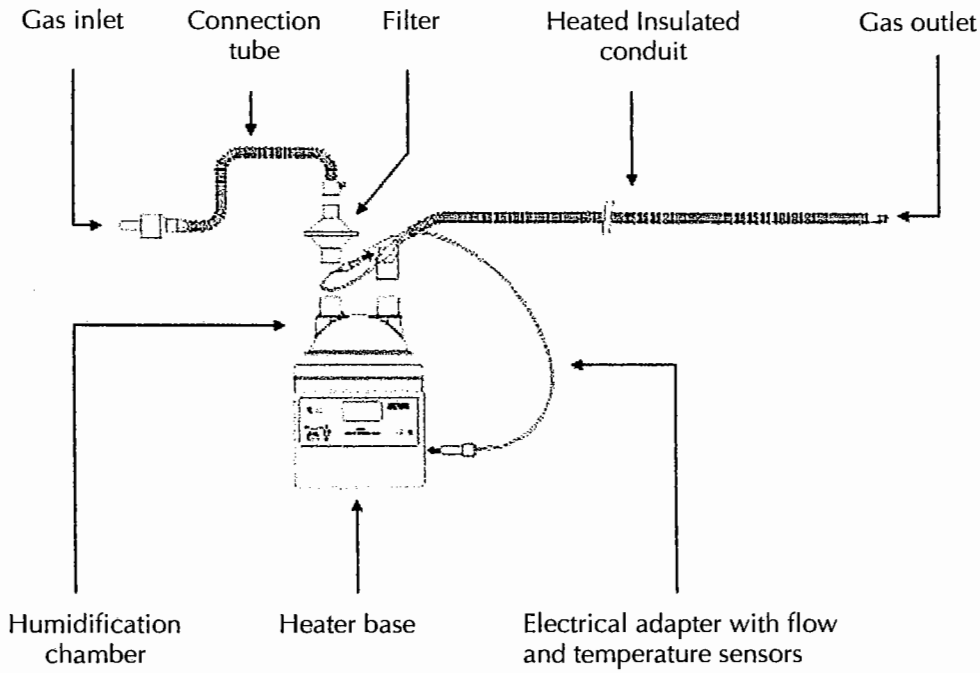

FIGURE 2. The humidification system, which has three components: a heater base; an electrical adapter with flow and temperature sensors; and a disposable set consisting of a connection tube, filter, and humidification chamber and heated insulated conduit. The system is compatible with all insufflators.

[RH]). Temperature and flow sensors at the outlet of the chamber ensure the temperature and humidity of the gas are controlled for the working flow range. The gas (37° C, 100% RH) then enters the conduit. To prevent loss of humidity and temperature in the conduit, it is heated by an internal heater wire, and the tubing is insulated. The temperature at the gas outlet is elevated slightly above body temperature to allow for cooling within the cannula so that the gas entering the abdominal cavity is saturated at body temperature. The heated insulated conduit is controlled by the heater base.

The temperature and humidity output at the tip of the Veress needle (34° C, 100% RH) was confirmed through in vitro experiments. This temperature was slightly lower than that observed in previous in vitro studies (35°–37° C) where a cannula was used rather than a Veress needle. The standard cold, dry CO_2 (20° C, 0% RH) was delivered to the control animals. The abdomens were insufflated at a flow of 1 L per minute (WISAP 7050E Op-Pneu Electronic, Sauerlach, Germany) through the right subcostal needle at a pressure of 8 mm Hg for 50 minutes (total of 50 L of gas per animal), and the gas exited through the other needle.

On completion of the insufflation, the abdomen was gently compressed to remove as much of the residual gas as possible, and 10 mL/kg of 0.9% saline was administered intravenously. The right subcostal insufflation needle was removed, and the cradle elevated to a head-up vertical position. Anteroposterior (AP) and right lateral radiographs were taken to confirm the removal of residual gas. A 20 mL bolus of gas corresponding to the experimental group was injected into the abdomen through the remaining needle. The needle was then removed, and the abdominal wall was sutured to prevent the leakage of the gas. A series of radiographs (AP and lateral) were taken at 5, 15, 30, 45, and 60 minutes (Figure 3). The animal remained in the vertical position throughout this time. Blood samples (2 mL) were collected just before insufflation (t=0), then at 2, 4, and 5 hours. After the radiographs were taken, the piglets were laid horizontal. Animals were killed by an overdose of intravenous barbiturate, and tissue samples were taken (diaphragm, intestine, liver, and spleen). The samples were fixed in 10% neutral buffered formalin, paraffin embedded, and stained with hematoxylin-eosin for histological examination by light microscopy.

Measurement of Outcomes

The AP and lateral gas-bubble radiographic images were traced onto white paper and analyzed using the Matlab (The Mathworks, Inc., Natick, MA) program to calculate the area of each gas-bubble profile. Blood samples were analyzed for IL-1β and TNFα using respective porcine ELISA kits (Quantikine, R&D Systems, Minneapolis, MN). Histological tissue sections were examined by light microscopy. The mesothelial surface on a variety of viscera, including liver, spleen, gut, and diaphragm, was examined from both groups.

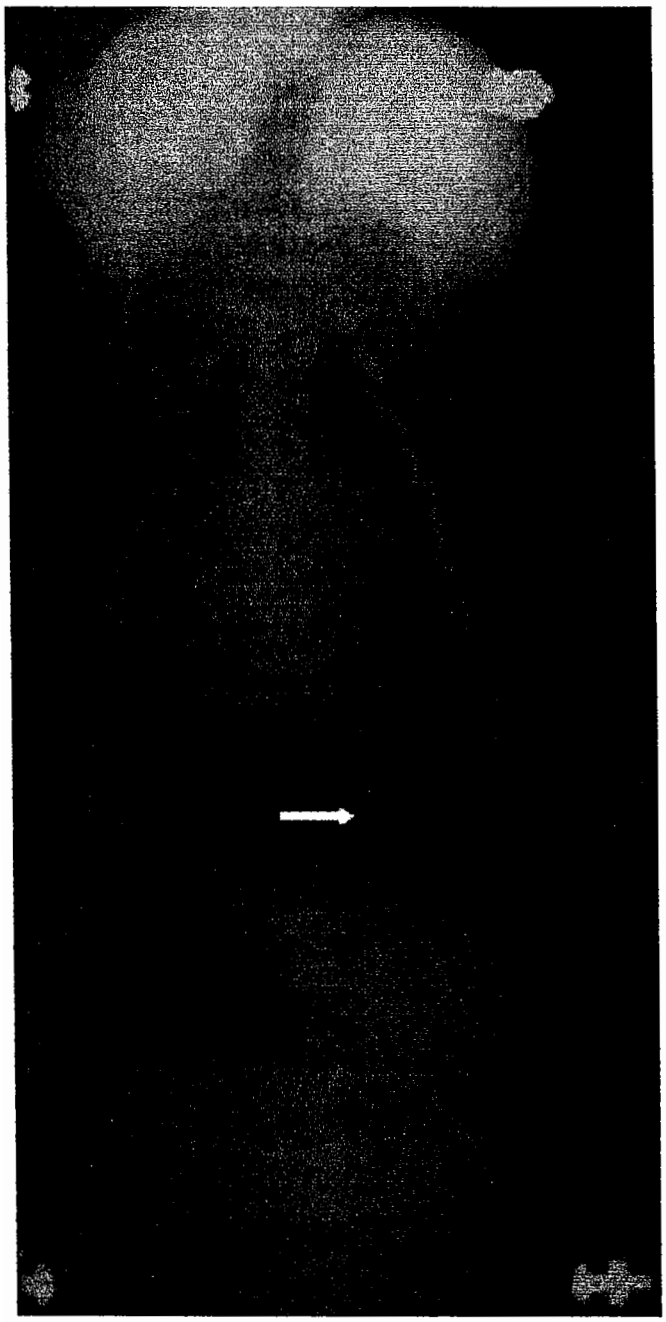

FIGURE 3. An anteroposterior radiograph of an anesthetized piglet, positioned vertically in a restraint device, at 5 minutes after insufflation with 20 mL of humidified, warmed CO_2. The white arrow indicates the CO_2 gas bubble under the diaphragm. The black arrow indicates normal gas in the stomach.

Statistical Analysis

The AP and lateral gas-bubble areas were analyzed by a mixed-model analysis of variance using the computer program Stata V7 (StataCorp, LP, College Station, TX). A random effect was entered for each pig, to allow for the possibility that the within-pig variation was different to the between-pig variation. As well as an effect for time, an effect for time^2 was fitted to allow for some leveling off in the response with time.

A two-sample *t* test assuming equal variances was used to compare the mean concentration of TNFα for each group at each time point. In addition, a repeated measures analysis of variance was to compare the changes in TNFα concentrations over time between the two groups.

Results

Area of Residual Gas Bubble

Figure 4 is a plot of mean gas-bubble area (mm^2) versus time (minutes) for both lateral and AP gas images. There was a significant reduction in area with time, with the humidified group having a more rapid reduction ($p < .05$) than the nonhumidified group and a significantly different curvature for the decrease. However, there was no significant difference between the means at any particular time point.

Cytokines

Both the humidified group and the nonhumidified group displayed similar increases in serum TNFα at 2 hours postincision (Figure 5). However, the TNFα levels in the humidified group began to fall after this, whereas the levels in the nonhumidified group remained elevated. When the linear relationship between the TNFα concentrations over time was compared between the groups, there was a significant difference ($p < .001$). The differences in plasma concentrations at any individual time point, however, did not reach statistical significance. In all but two piglets (one from each group), the serum levels of IL-1ß were below the threshold of detectability. Of the two that did demonstrate an IL-1ß response, piglet A (nonhumidified) demonstrated the largest and most sustained response (up to 160 pg/mL at 5 hours). Piglet B (humidified) had a relatively more moderate rise at 4 hours (37 pg/mL), which subsided to 9 pg/mL at 5 hours. A previous pilot study had indicated that there was no detectable IL-6 response to either treatment during the timeframe in which the blood samples were taken.

Histology

The mesothelial surface was examined by light microscopy, and no discernible difference was noted between the two groups. Foci of mesothelial nuclear prominence and cellular absence were noted, but this was found in both groups and may have been an artifact. Wrinkling of the mesothelial surface was noted only on the gut, and that may be the result of muscular contraction following death. It was not seen on solid organs. The time interval from the end of the experiment to the killing of the animal may have been too short for any damage incurred by the cells to be distinguished by light microscopy.

Discussion

This study shows that the use of prehumidified CO_2 for insufflation significantly accelerates both the rate of elimination of residual intraperitoneal gas pocket and the rate of decline of serum TNFα in the postoperative period.

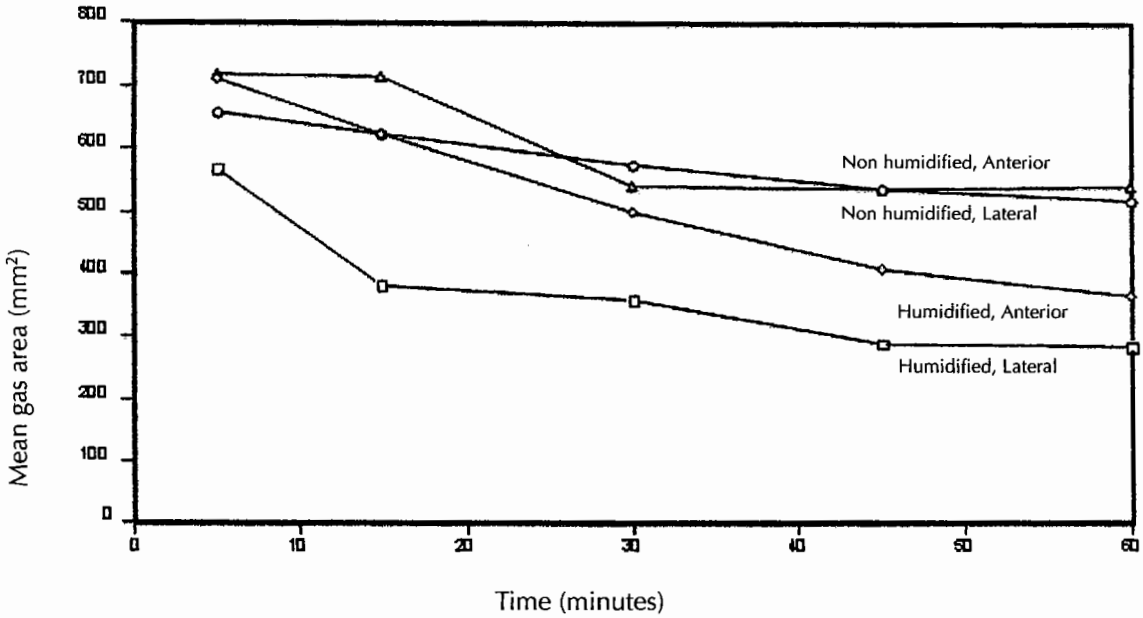

FIGURE 4. Mean gas-bubble area (mm^2) versus time (minutes) for both lateral and anteroposterior gas images. There was a significant reduction in area with time with the humidified group having a more rapid reduction ($p < .05$) than that the nonhumidified group. Error bars are omitted for reasons of clarity; n=5.

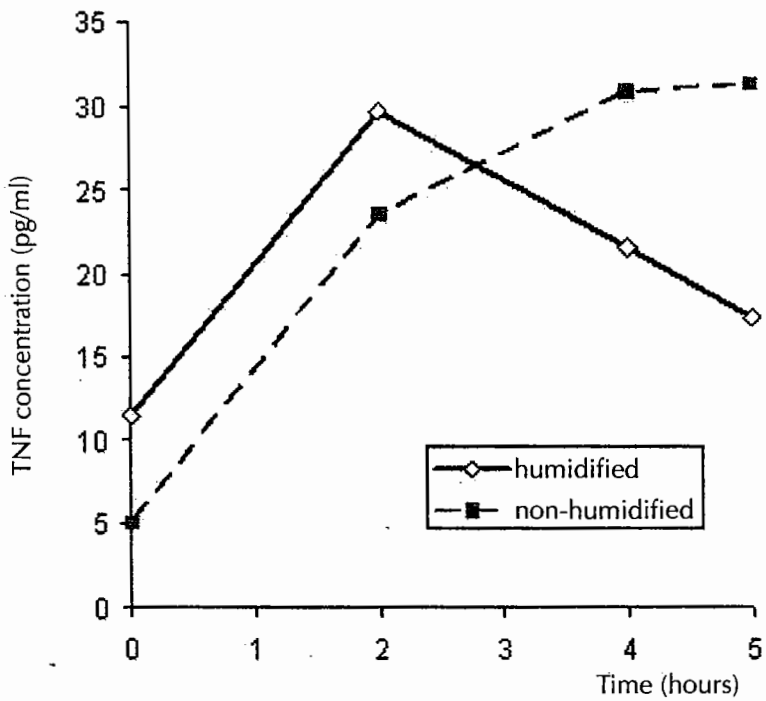

FIGURE 5. Serum TNFα concentrations over time following insertion of laparoscope and insufflation of the abdomen with either humidified or dry CO_2. There is a significant difference ($p < .001$) in the linear relationship of TNFα concentrations over time between the groups. Error bars are omitted for reasons of clarity; n=5.

Previous studies have used radiographic methods to determine the duration of postlaparoscopic residual pneumoperitoneum.[12,15–17] However, to our knowledge, this is the first evidence that the resolution of the residual pneumoperitoneum can be accelerated and that this can be achieved by using prehumidified CO_2 for insufflation.

It has traditionally been considered that during laparoscopic procedures "warming and humidifying insufflation gas is intended to decrease heat loss."[18] There is some debate in the literature as to whether there are clinical benefits of using humidified CO_2 to reduce hypothermia, particularly for short procedures. However, it should be recognized that the hypothermia is a result of the evaporation of water into dry gas, rather than a direct effect of gas temperature, and that this water evaporation has a number of potential consequences in addition to hypothermia.

In the current study, serial radiographs demonstrated significantly faster removal of residual gas in the humidified arm of the study. This could be clinically beneficial, since there is considerable evidence to suggest that reducing the size of the gas bubble will lead to a reduction in postlaparoscopy pain.[12–14]

The faster resolution of the residual pneumoperitoneum observed in this study implies faster diffusion of CO_2, which in turn depends on how fast it is dissolving. Since CO_2 gas is highly soluble in water, this implies that the use of humidified gas preserves the moist milieu of the serous fluid lining of the peritoneal cavity. This is consistent with the previous findings showing that humidification preserves the viscosity of the peritoneal fluid.[19] The preserved peritoneal lining would allow CO_2 to dissolve faster and lead to a faster reduction in the amount of residual gas.

Previous clinical studies showing that the use of humidified CO_2 reduces postoperative pain, especially shoulder tip pain,[9,11,20,21] may be explained, in part, by rapid dissolution of CO_2 across an undamaged peritoneal surface. Although further work is required to confirm the direct causal link between the findings presented here and reduction of pain in the clinical situation, it opens up the possibility that more rapid removal of CO_2 can be achieved without the need for abdominal gas drains to be left in situ following the operation. This would offer several advantages. Although abdominal gas drains[13] and/or intraperitoneal saline infusions[14] following pneumoperitoneum reduce the postoperative pain associated with residual gas, they do not correct any damage already present due to desiccating nature of the dry gas. Furthermore, they may increase the likelihood of infection, and their removal necessitates an additional manipulation for the medical staff and the patient.

Desiccation of the peritoneal tissue and subsequent complications due to dry CO_2 is a recognized problem that should theoretically be reduced using humidified gas.[1–3] However, there are very few in vivo studies to date that make a direct comparison of the effects of dry and humidified CO_2 on the peritoneal surface. Direct postsurgical examination of the peritoneal surface in human patients is clearly impractical; but serum cytokine levels can be used to give an indication of the inflammatory response, and there is some evidence to suggest that humidified CO_2 reduces this.[6]

In the present study, both groups had a similar rise in serum TNFα within the first 2 hours. It is probable that this was due to the initial procedures necessary for the study (i.e., anesthesia, cannulation, incision into abdominal wall) and the increase in pressure within the abdomen. Following the initial response, the piglets insufflated with humidified CO_2 underwent a decline in serum TNFα levels, suggesting that there was no damage occurring following the procedures outlined above. In contrast, the plasma concentrations of TNFα in the nonhumidified group increased between 2 and 4 hours and remained elevated at 5 hours. Also, in the two piglets that did demonstrate an IL-1ß response, the plasma profile of IL-1ß in the piglet in the nonhumidified group remained elevated, in contrast to that seen in the piglet in the humidified group.

These findings suggest that after the initial surgery that was common to both treatment groups, there was some other factor that was sustaining TNFα release in the nonhumidified group. Whether this factor was desiccation of the tissue due to evaporation, or prolonged residence of the CO_2 causing distension or pH changes is yet to be determined. However, the data suggest that the use of humidified CO_2 in place of dry CO_2 prevents continuing tissue damage following insufflation of the abdomen.

In this study, no structural damage of the peritoneum was detected using either humidified or dry CO_2. In contrast, it has been found that humidified and heated CO_2 in rats caused peritoneal tissue damage to the same extent as dry CO_2.[22] However, in that study anesthesia was induced and maintained with repeated intraperitoneal injections of pentobarbital sodium. Thus, some of the observed damage could be attributed to the direct effect of pentobarbital on the peritoneal surface. It is possible that no damage occurred in our study due to the conservative conditions used, although differences between species should be considered. Electron-microscopic investigations have shown that insufflation of dry CO_2 causes ultrastructural damage of the cells lining the peritoneum in mice,[23] whereas humidification offered protection of the similar cells lining the pleura in pigs.[9] Clearly more studies, preferably using inhaled rather than intraperitoneal anesthesia, as well as using more-sensitive methods such as electron microscopy, are required to clarify this issue.

The selection and use of animal models for this type of study confer a number of advantages and disadvantages compared with the use of human patients. More intensive/invasive sampling and monitoring is possible using animal models, but the more subjective outcomes such as pain, recovery time, etc., are difficult to determine. The piglet was selected as the most appropriate model for this study. This species has anatomic similarity with humans and is large enough for radiographic imaging, yet at this age reasonably cooperative and readily handled. The use of this model indicates that humidified CO_2 offers a number of potential advantages over dry CO_2. Further studies in patients are required to determine the level of clinical benefit.

Conclusion

Following pneumoperitoneum, residual intraabdominal CO_2 dissipates more rapidly when the gas is heated and humidified compared to cool and dry. This is associated with a reduction in the duration of the inflammatory response measured by TNFα production. These effects may contribute toward a reduction in postlaparoscopic pain and other complications.

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