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Effects of Warmed and Humidified CO_2 Surgical Site Insufflation in a Novel Experimental Model of Magnetic Compression Colonic Anastomosis

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Abstract

Background. Pneumoperitoneum insufflation with warmed and humidified carbon dioxide (WH-CO_2) can prevent heat loss and increase tissue oxygenation. We evaluated the impact of localized WH-CO_2 insufflation on the anastomotic healing process. *Methods*. Sixty male Wistar rats were randomized: Group 1 (control, n = 12), Group 2 (cold and dry CO_2, CD-CO_2, n = 24), and Group 3 (WH-CO_2, n = 24). A magnetic compression side-to-side colonic anastomosis was performed under 60-minute local abdominal CO_2 flow insufflation. Animal temperature was recorded. IL-1, IL-6, and CRP levels were assessed before and after insufflation and on postoperative day (POD) 7 and POD 10. Endoscopic follow-up was performed on POD 7 and POD 10. A burst pressure (BP) test of the specimen was performed on POD 10, and histopathological analysis was then performed. Metabolomics of the anastomotic site was determined. *Results*. Seven rats (5 CD-CO_2 group, 1 WH-CO_2 group, and 1 control group) died during the survival period. Necropsies revealed intestinal occlusions (n = 2). One additional rat from the CD-CO_2 group was sacrificed on POD 7 due to intestinal perforation. The postoperative course was uneventful in the remaining cases. There was no difference in BP among the groups. Thermal monitoring confirmed that WH-CO_2 insufflation was effective to reduce heat loss. IL-1 levels were statistically and significantly lower on POD 10 in the WH-CO_2 group than the CD-CO_2 group but not lower than the control group. CRP levels, histopathology, and metabolomics did not show any difference between the 3 groups. *Conclusions*. WH-CO_2 was effective to preserve core temperature. However, it did not improve anastomotic healing.

Keywords

CO_2 insufflation, colorectal anastomosis, magnetic anastomosis, colonoscopy, anastomotic leak, experimental model, metabolomics profiling

Introduction

In minimally invasive surgery, the working space is obtained with controlled insufflation of carbon dioxide in the peritoneal cavity. Gas flow may induce heat loss and tissue desiccation. The potential beneficial impact of peritoneal insufflation with warmed and humidified CO_2 (WH-CO_2) during abdominal surgery has been a matter of debate for decades. Some studies suggest that there is a variety of systemic and local positive effects of WH-CO_2, including a reduced risk of hypothermia,[1-3] reduced postoperative pain,[4] augmented tissue oxygenation,[5] reduced adhesion formation, a reduced risk of peritoneal tumor cell implantation,[6,7] reduced peritoneal

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desiccation,[8] and a reduced risk of surgical site infections.[2] In a recent experimental study, Marshall et al[5] demonstrated a significant increase in tissue oxygenation induced by the local insufflation of WH-CO_2 in a rodent model of open abdominal surgery. Bowel perfusion is an important factor which improves gastrointestinal anastomosis healing.[9] Our hypothesis was that WH-CO_2, besides preventing the occurrence of intraoperative hypothermia, will also increase local bowel perfusion and reduce the risks of anastomotic leakage. Few studies have assessed the impact of CO_2 insufflation in colorectal surgery[10,11] but none with the specific aim to study the effects of WH-CO_2 on the healing process of colorectal anastomosis. Our aim was to evaluate the systemic and local impact of WH-CO_2 laminar insufflation in a survival rodent model of magnetic compression colonic anastomosis.

Materials and Methods

Animals

Animal experimentations were performed in accordance with the European recommendations (Directive 2010/63/UE, September 22, 2010) and the French regulations (Décret 2013–118, February 1, 2013) and received the approval from the local ethical committee on animal experimentation (ICOMETH, protocol number 38.2015.01.073) and the French Ministry of Superior Education and Research (MESR, Authorization No. APAFiS #2834).

A total of 60 Wistar rats (mean weight 563 ± 56 g) were used. Experimentations were performed over a 4-month period, dividing the population into 2 groups which significantly differed in terms of weight due to the running experimental time (Protocol 1: n = 30 Wistar rats, 523 ± 9 g; Protocol 2: n = 30 Wistar rats, 603 ± 5 g). Animals were randomized into 3 groups. All 3 groups underwent the same surgical procedure. Group 1 (control, n = 12) was operated on in ambient air. Group 2 (CD-CO_2, n = 24) was operated on using cold and dry CO_2 insufflation. Group 3 (WH-CO_2, n = 24) was operated on using warmed and humidified CO_2 insufflation (Figure 1).

The sample size was calculated based on data on the healing of digestive anastomosis in rodents available in the literature.[12] The primary chosen outcome was the burst pressure (BP). Using a superiority design and fixing an increase of BP equal to 20 mmHg in the experimental arm as the limit of superiority, a sample size of 12 animals per group was considered to be sufficient to detect a significant difference with an α at 10% and a power (1-β) of 80%.

The animals had free access to food and water before and after surgical procedures. All experimental procedures were performed under 1 to 3% isoflurane anesthesia delivered by a mask, with a 2 to 3 L/min O_2 flow rate. Animals were operated on at a fixed room temperature (22°C) and were placed in a supine position into a plastic box, in order to limit CO_2 diffusion to a defined volume. To prevent animals from breathing CO_2, a leak-proof hole was made in the box and the head was introduced directly into the anesthesia mask.

The rats survived for 10 days after the surgical procedure and underwent anesthesia on postoperative days (PODs) 7 and 10 for follow-up tests. During the survival period, the animals were monitored daily to detect the occurrence of adverse events. At the end of the experiments, the animals were euthanized by performing an aortic section.

Bowel Preparation and Colonoscopy

Following induction of anesthesia, a warm saline solution enema was administered prior to colonoscopy. A flexible intubation fiberscope (3.7 mm outer diameter with 1 working channel, KARL STORZ, Tuttlingen, Germany) was used to perform the colonoscopy. The fiberscope was connected to a portable all-in-one Tele Pack system (KARL STORZ, Tuttlingen, Germany), which was the interface for the procedure and allowed the recording and storage of all images. No gas was insufflated during the procedure. Bowel exploration was made possible by injecting a saline solution.

Ten procedures were performed using a 2.8-mm flexible video ureteroscope (KARL STORZ, Tuttlingen, Germany) connected to a laparoscopic image 1 HD system (KARL STORZ, Tuttlingen, Germany).

Surgical Procedure

A single surgeon helped by a second operator performed all procedures. The abdominal area was shaved in a supine position and a full-thickness of 7-cm sagittal median incision was made. Gas insufflation started immediately after abdominal cavity opening. A self-retaining retractor was placed on the abdomen to divaricate the cavity. A surgical exploration of the cavity was performed, and the descending colon was identified. A magnetic side-to-side anastomosis was created adopting a 2-cm colonic loop model (see paragraph below). The average surgical time was 20 minutes. After a total insufflation of 60 minutes, mass closure was performed using a 3/0 monocryl suture.

Gas Insufflation

In the WH-CO_2 group, warmed and humidified CO_2 was delivered by a HumiGard™ humidifier equipped with VITA-diffuser™ catheters (Fisher & Paykel, New Zealand). The adopted insufflation system humidified the CO_2 gas by conditioning it into a water-filled chamber seated on a heating plate. Once the heater plate is warmed to the

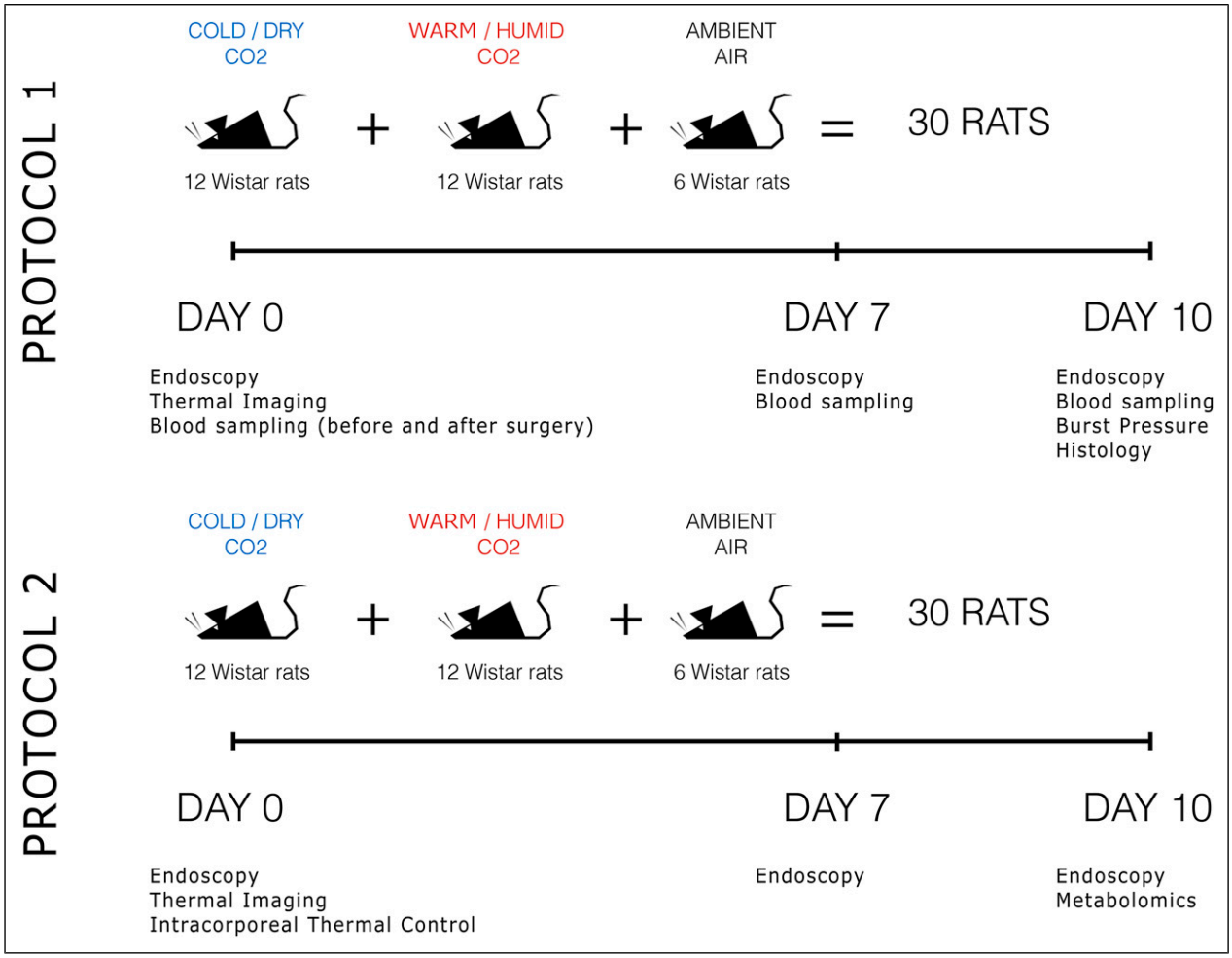

Figure 1. Timeline and follow-up. The experiment was divided into 2 phases. The difference was that in the first 30 rats, the anastomosis was sent for histopathological analysis, and in the 30 other rats, anastomosis underwent metabolomics profiling.

target temperature, the heated water in the chamber produced vapor that humidified the CO2 gas as it passed through the chamber. The gas flow provided was set at 10 L/min while the temperature and the relative humidity were automatically determined by the instrument. In the CD-CO_2 group, cold and dry CO_2 was delivered by the same system disconnected from the power supply. The gas flow was identical. In the control group, no gas was insufflated.

Colonic Loop Model and Magnetic Compression Anastomosis

A model of magnetic compression anastomosis was specifically adopted in order to reduce the risk of bias given by the surgeon's expertise and the learning curve while performing a hand-sewn anastomosis. In addition, the adoption of a loop model eliminated the need for a surgical dissection and vascular ligation, hence reducing the risk of methodological bias.

Two neodymium round magnets (2 mm height/4.12 N strength), 4 mm in diameter, were adopted considering the caliber of the rats' colons. After a 2-cm descending colon tract was measured and highlighted with a surgical marker (Covidien, Dublin, Ireland), the first magnet was endoscopically driven to the proximal anastomotic site, and after kinking of the colon using atraumatic plastic forceps, the second magnet was delivered to the distal anastomotic site. The coupling of the magnets created a compression side-to-side anastomosis, whereas the colonic loop worked as a fecal bypass in order to allow the passage of stools during the anastomotic completion time (Supplementary Video 1).

Perioperative Temperature Monitoring

The animal temperature was monitored using a FLIR i5 thermographic camera (FLIR Systems, Wilsonville, Oregon, US). Two regions of interest (ROIs) were evaluated. ROI 1 included the externally exposed abdominal

surface, whereas ROI 2 included the animal skin surface. Thermographic photos were captured every 10 minutes after starting the insufflation. Data were exported using FLIR tools software (FLIR Systems, Wilsonville, Oregon, US) and analyzed using MATLAB software (MathWorks, Inc, Massachusetts, US) through a specifically designed algorithm (Figure 2). The algorithm extrapolated temperature values from the images and evaluated the mean temperature value and the associated standard deviation within each selected ROI.

Additionally, a thermocouple system was adopted to assess intraluminal bowel temperature and abdominal cavity temperature. Two probes (1 mm in diameter) were connected to a recording monitor (Yokogawa FX 1006). The first probe was placed deep inside the open abdominal cavity, whereas the second probe was inserted transanally with the distal tip proximal to the anastomotic site. Temperature values were continuously recorded (every 2 seconds) starting from 20 minutes to the end of the procedure (Figure 3). The delay time in procedure recording was due to the endoluminal procedure completion time.

Endoscopic Follow-up

The endoscopic follow-up of the anastomotic site was performed on POD 7 and POD 10, repeating endoscopies under anesthesia. The procedure was carried out as previously described.

All endoscopic procedures were performed with the same flexible intubation fiberscope used during surgical procedures with the exclusion of 30 follow-up cases, which were performed using a rigid 4-mm diameter 30-degree arthroscope (KARL STORZ, Tuttlingen, Germany) connected to the Tele Pack system (KARL STORZ, Tuttlingen, Germany). Water injection during the procedure was possible through a tailored angiographic cannula placed around the instrument.

Endoscopic assessment of the anastomosis was performed by 2 surgical residents (F.M. and P.R.) and a senior surgeon (S.K), all experienced in surgical endoscopy.

Plasmatic Markers of Inflammation

Under anesthesia, blood samples were collected from animals' tails in Vacutainer tubes (Becton, Dickinson,

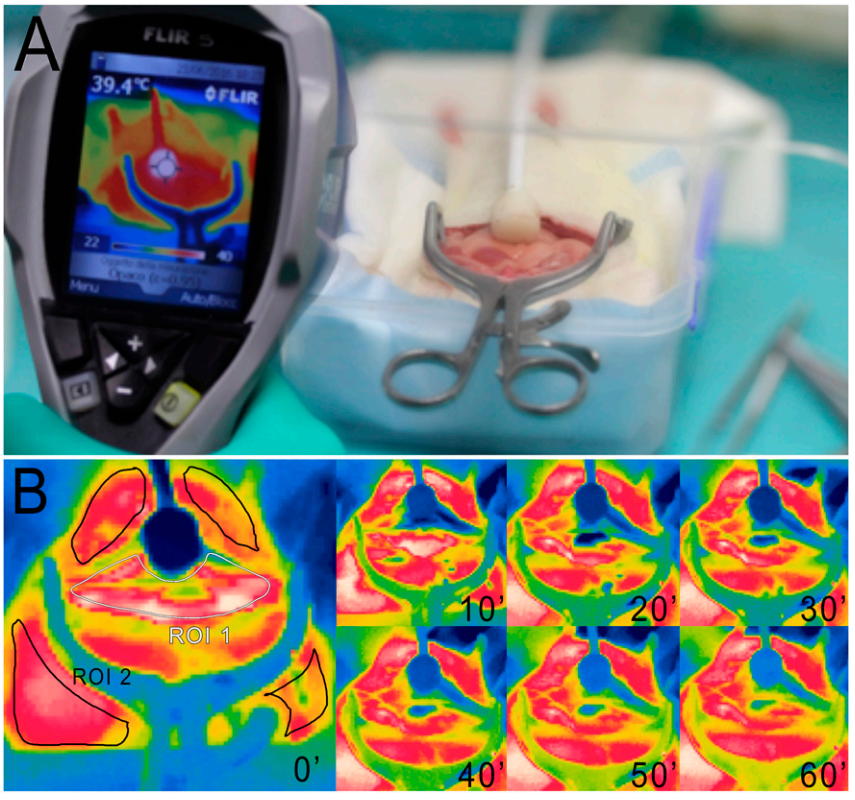

Figure 2. Thermography. (A) Thermal images were recorded using an infrared camera. (B) Using a dedicated algorithm in MATLAB, the temperature was analyzed in 2 regions of interest: ROI 1 included the externally exposed abdominal surface, and ROI 2 included animal skin. Thermographic images were captured every 10 minutes after insufflation.

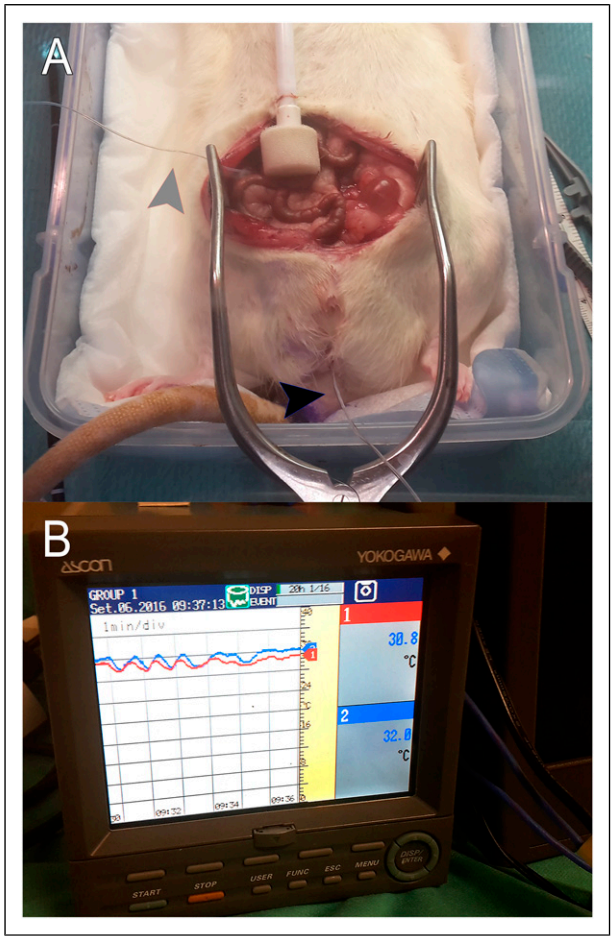

Figure 3. Abdominal cavity and intraluminal bowel temperature monitoring. (A) Abdominal cavity temperature and intraluminal bowel temperature were recorded with a thermocouple-based system. The first thermal probe (gray arrowhead) was placed inside the open abdominal cavity, and the second thermal probe (black arrowhead) was transanally inserted at the distal tip proximal to the anastomotic site. (B) Temperature recorded by the probes were shown and recorded with a dedicated 4-channel monitor.

Oxford, United Kingdom) at 4 different time points: prior to surgery, after surgical procedure completion, on POD 7 and on POD 10. Interleukin-1 (IL-1), Interleukin-6 (IL-6), and C-reactive protein (CRP) were dosed on plasma samples by means of enzyme-linked immunosorbent assay (ELISA) for rats (pg/mL).

Burst Pressure Measurement

After the sacrifice of the first 30 animals (Protocol 1), a colon loop was resected, cleaned in saline solution, and sutured on both sides to close the fluid circuit controlled by an arterial blood pressure sensor connected to an operating room hemodynamic monitoring system (General Electric Healthcare Company, Massachusetts, US). The pressure inside the system was increased until the bursting pressure was reached. All the values recorded on the screen were saved and exported for analysis (Supplementary Video 2).

Histological Examination

After the macroscopic examination and BP test, anastomotic specimens were fixed in 4% formaldehyde for histological examination. Specimens were embedded in paraffin and sectioned at a 4 µm thickness. Hematoxylin–eosin staining was performed. A pathologist applied a semi-quantitative histology score to evaluate the anastomosis in terms of local inflammation. The percentage of the specimen surface covered with inflammatory cells was measured and scored as follows: 0 = no inflammation, 1 = mild inflammation (occupied surface 1%–30%), 2 = moderate inflammation (occupied surface 31%–60%), and 3 = severe inflammation (occupied surface > 60%). The same score system was used to evaluate the extent and severity of fibrosis, congestion, edema, and neovascularization.

Metabolomics

In the last 30 animals (Protocol 2), after the sacrifice on POD 10, the colonic loop was resected, placed in an Eppendorf tube, and snap frozen in liquid nitrogen. In these samples, metabolomics profiling of the anastomotic site was determined using high-resolution magic angle spinning (HRMAS) nuclear magnetic resonance spectroscopy. HRMAS spectra were recorded on a spectrometer (Bruker Advance III 500) equipped with a 4-mm double resonance (1H, 13C) gradient HRMAS probe, as previously described.[13,14]

Statistics

GraphPad Prism software (GraphPad Software Inc, La Jolla, CA, USA) and MedCalc software were adopted for statistical analysis. The Student's t-test was used to calculate P values for continuous variables and Kruskal–Wallis ANOVA for multiple analyses. A P value $< .05$ was considered statistically significant.

Results

No intraoperative deaths occurred. Seven rats died during the postoperative follow-up. Two deaths occurred due to intestinal occlusion. An additional rat was actively sacrificed on POD 7, after endoscopic control because of the presence of a peri-anastomotic perforation. The remaining necropsies were negative (Table 1). Mortality rate between CD-CO2 (n = 6) vs WH-CO2 (n = 1) vs control groups (n = 1) was not statistically significant (Fisher's exact test: $P = .09$, in both cases).

No other postoperative complications occurred. All surviving animals resumed bowel movements during the first 48 hours, demonstrating a good colonic loop function. Adhesions around the anastomotic site were observed in all animals at explorative laparotomy, but no macroscopic signs of leakage were observed.

Endoscopy

Fifty-three animals underwent an endoscopic follow-up on POD 7, and only 52 survived until endoscopic follow-up on POD 10. All these animals expelled the magnets. No intestinal occlusion occurred with the colon loop allowing for the passage of stools. Thirty-four animals expelled the magnets on POD 7 or before, and 5 of them expelled the magnets during endoscopic procedures. The remaining 18 animals expelled the magnets on POD 10 or before, and 5 of them expelled the magnets during endoscopic procedures. No difference was shown between the 3 groups (Supplementary Video 1).

Burst Pressure

The mean BP was 205.3 ± 26, 204.0 ± 38, and 205.0 ± 21 mmHg in the WH-CO_2 group, CD-CO_2 group, and control group, respectively. All specimen ruptures were located in the proximal or distal colon, but no ruptures occurred on the anastomotic site. There was no significant difference between the 3 groups.

Histology and Immunohistochemistry

Results are shown in Figure 4. No statistical differences were shown in the scale of mean global inflammation, fibrosis, edema, and neovascularization of the specimens. There were no statistical differences when the results were analyzed to isolate the effect of CO_2 insufflation rather than temperature exposure.

Systemic Inflammation

Blood analysis performed in animals belonging to protocol 1 is shown in Table 2.

Table 1. Postoperative Adverse Events.

	Animal Number	Group Treatment	Postoperative Day	Death/Sacrifice	Necropsy
Protocol 1	1	Control	5	Death	Negative
	2	CD-CO_2	5	Death	Bowel occlusion
	16	CD-CO_2	3	Death	Bowel occlusion
	30	CD-CO_2	7	Sacrifice	Bowel perforation
Protocol 2	31	CD-CO_2	1	Death	Negative
	50	CD-CO_2	7	Death	Negative
	58	WH-CO_2	2	Death	Negative
	59	CD-CO_2	0 (after surgery)	Death	Negative

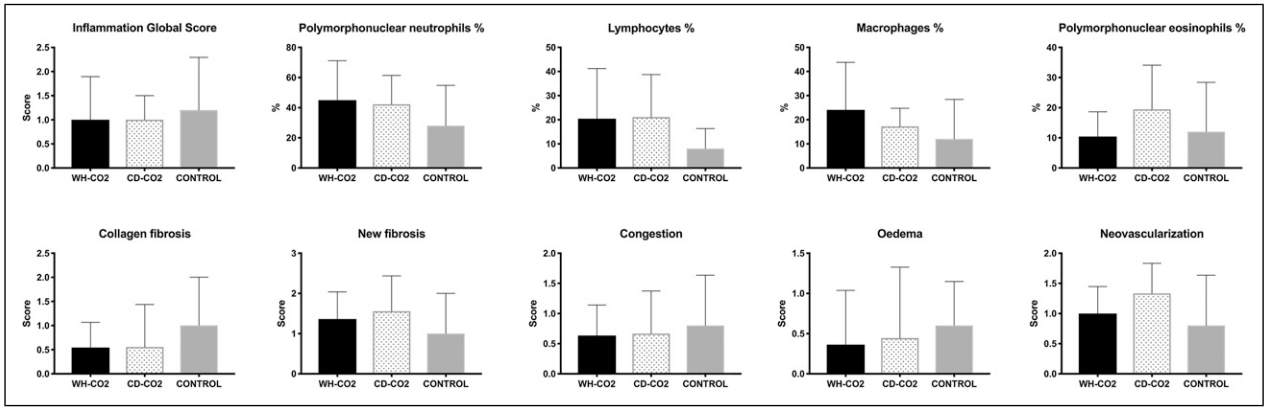

Figure 4. Histology scores. The percentage of the specimen surface covered with inflammatory cells was measured and scored as follows: 0 = no inflammation, 1 = mild inflammation (occupied surface 1 to 30%), 2 = moderate inflammation (occupied surface 31 to 60%), and 3 = severe inflammation (occupied surface >60%). The same score system was used to evaluate the extent and severity of fibrosis (collagen or new), congestion, edema, and neovascularization.

The interleukin-1 levels statistically differed on POD 10 between CD-CO_2-treated animals and WH-CO_2-treated animals ($P < .05$). No other differences were shown between the groups at any given time. Mean CRP levels did not differ between the 3 groups at any time.

Table 2. Inflammatory Markers.

Parameter	Control	CD-CO_2	WH-CO_2
IL-1 (pg/mL)			
Before surgery	184.9 ± 23.4	186.4 ± 17.7	189.7 ± 32.4
After surgery	195.4 ± 17.7	208.0 ± 37.7	209.3 ± 46.3
POD 7	179.9 ± 28.1	195.7 ± 31.5	188.6 ± 27.3
POD 10	201.8 ± 55.5	243.1 ± 83.7	188.0 ± 28.0
CRP (pg/mL)			
Before surgery	23.7 ± 24.2	23.8 ± 7.9	24.8 ± 6.5
After surgery	37.7 ± 6.4	34.8 ± 11.5	39.2 ± 9.1
POD 7	30.1 ± 6.0	29.8 ± 8.1	31.6 ± 8.9
POD 10	30.4 ± 9.8	34.5 ± 7.3	30.8 ± 7.9

Abbreviations: IL, interleukin; CRP, C-reactive protein; POD, postoperative day.

Interleukin-6 analysis results were omitted because nonhomogeneity maybe due to a technical error when performing the ELISA test.

Thermal Control

The mean abdominal surface temperature (ROI 1) recorded at the starting point was 31.2 ± 1.7°C, 29.1 ± 2.0°C, and 30.7 ± 1.2°C in the WH-CO_2 group, CD-CO_2 group, and control group, respectively. The difference between the WH-CO_2 group and the CD-CO_2 group was statistically significant ($P < .05$) since the beginning of the insufflation and during the experiment ($P < .01$) with a final temperature of 33.4 ± 1.9°C, 24.1 ± 2.0°C, and 27.7 ± 1.5°C in the WH-CO_2 group, CD-CO_2 group, and control group, respectively. A significant difference was found at 10, 30, 50, and 60 minutes when comparing WH-CO_2 insufflation vs no insufflation of the control group (Figure 5A).

The temperature differs statistically between the WH-CO_2 group and the CD-CO_2 group ($P < .05$), also analyzing mean skin temperature (ROI 2) which was 31.5 ± 1.3°C, 29.9 ± 1.3°C, and 31.0 ± 1.2°C at the beginning of

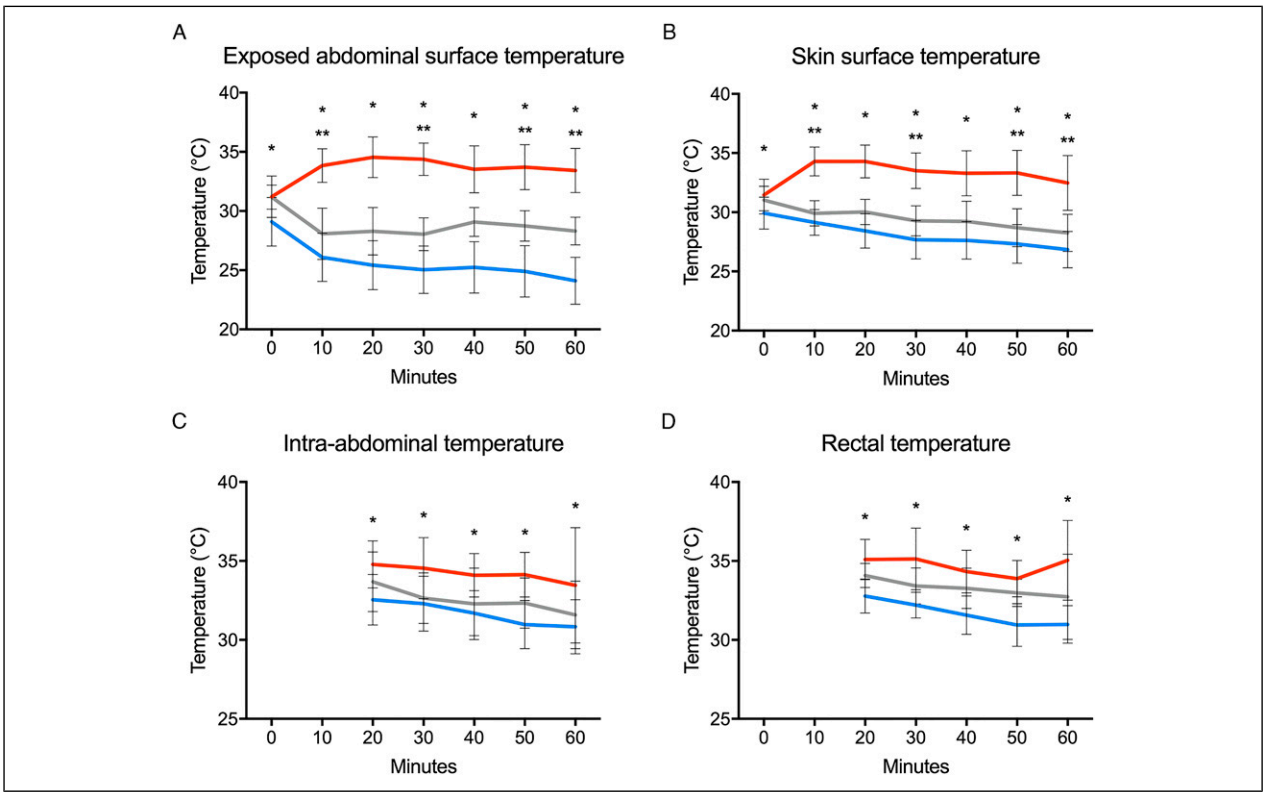

Figure 5. Temperature evolution over time. (A) Exposed abdominal surface temperature. (B) Skin surface temperature. (C) Intra-abdominal temperature. (D) Rectal temperature. (*) significant difference between WH-CO_2-treated rats and CD-CO_2-treated rats; (**) significant difference between WH-CO_2-treated rats and the control group.

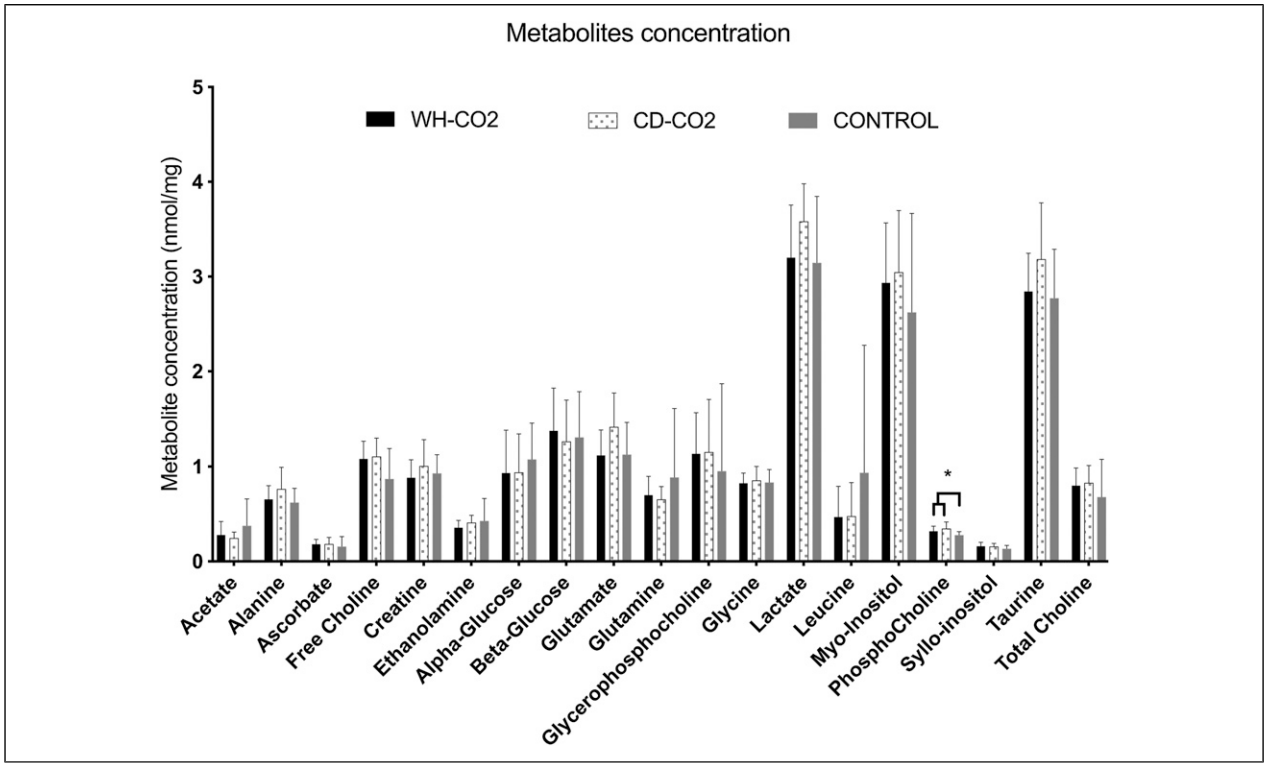

Figure 6. Metabolomics profiling of the anastomosis. A total of 19 metabolites were quantified in surgical specimens after sacrifice. No statistical differences were shown except for cumulative analysis where CO_2-treated animals showed significantly higher levels of phosphocholine than the control group.

the treatment in the WH-CO_2 group, CD-CO_2 group, and control group, respectively. At the end of the procedure, mean temperatures were 32.5 ± 2.3°C, 26.8 ± 1.5°C, and 28.3 ± 1.6°C in the WH-CO_2 group, CD-CO_2 group, and control group, respectively. A significant difference between the WH-CO_2 and the control group was evident at 10, 30, 50, and 60 minutes (Figure 5B).

The Mean intra-abdominal temperatures recorded at the starting point were 34.8 ± 1.5°C, 32.5 ± 1.6°C, and 33.7 ± 1.9°C in the WH-CO_2 group, CD-CO_2 group, and control group, respectively. These data showed a significant ($P < .01$) difference between WH-CO_2- and CD-CO_2-treated groups. The difference remained consistent until insufflation completion with a 60-minute recorded temperature of 33.4 ± 3.6°C, 30.8 ± 1.7°C, and 31.6 ± 2.1°C in the WH-CO_2 group, CD-CO_2 group, and control group, respectively. There was no difference between the WH-CO_2 group and control group (Figure 5C).

The mean rectal temperatures recorded at the starting point in the WH-CO_2 group, CD-CO_2, and control group were 35.1 ± 1.3°C, 32.8 ± 1.1°C, and 34.1 ± .7°C, respectively. Values were significantly different when comparing the WH-CO_2 group with the CD-CO_2 group. The difference remained significant ($P < .01$) during the entire procedure. The final temperatures recorded were 35.0 ± 2.5°C, 30.9 ± 1.2°C, and 32.7 ± 2.7°C in the WH-CO_2 group, CD-CO_2 group, and control group, respectively. No significant difference was evident when comparing the WH-CO_2 group with the control group (Figure 5D).

Metabolomics

A total of 19 metabolites could be quantified in anastomotic site tissue (acetate, alanine, ascorbate, free choline, creatine, ethanolamine, alpha-glucose, beta-glucose, glutamate, glutamine, glycerophosphocholine, glycine, lactate, leucine, myo-inositol, phosphocholine, scyllo-inositol, taurine, and total choline). No statistical differences were shown when comparing the 3 different groups (Figure 6).

Discussion

The effect of carbon dioxide insufflation during surgery was widely discussed in the literature. The use of this gas is considered the gold standard in minimally invasive surgery. It is under investigation in operative flexible endoscopy, although it does not play a standardized clinical role in open surgery. The potential benefits of warmed and humidified gas insufflation during open surgery remain controversial as in minimally invasive surgery.

A Cochrane analysis performed in 2011, which included 16 studies, failed to demonstrate any positive effects of heated gas insufflation, with or without humidification, in terms of postoperative pain, length of hospital stay, or postoperative complications.[15] A more recent systematic review has found substantial benefits regarding the use of WH-CO_2, with 7 human randomized controlled trials reporting lower postoperative pain.[16] Additionally, some investigations have suggested promising local effects in the abdominal cavity with augmented tissue oxygenation[5] and reduced inflammation and adhesion formation.[6] Humidified and warmed gas insufflation prevents intraoperative hypothermia during laparoscopic surgery,[16] which is *per se* a source of postoperative complications.[17,18] Intraoperative core temperature has been advocated as a potential modifiable risk factor for colorectal anastomotic leakage.[19] A clinical trial (NCT02975947) aiming to evaluate the impact on anastomotic leakage of WH-CO_2, among others, has been completed in Australia, but data are not available yet.

Based on such evidence, we have formulated the hypothesis that intraoperative WH-CO_2 insufflation could have promoted anastomotic healing by both preventing hypothermia and stimulating the local vascular supply. To the best of our knowledge, this is the first experimental study investigating the potential effects of WH-CO_2 on the healing process of colorectal anastomosis. In order to reduce technical related issues as much as possible and to achieve a standardized and reproducible anastomosis, we designed a novel experimental model (see Supplementary Video). We opted for an endoluminal magnetic compression loop anastomosis without resection to exclude the impact of the vascular supply on the healing process and isolate the effects of CO_2 insufflation. Magnetic compression anastomoses have been introduced in the 1980s and successfully tested in digestive and hepatobiliary sutureless anastomoses.[20] The magnets were introduced endoluminally and connected across the viscera walls to be anastomosed. Compression induces an ischemia–necrosis–scarring cycle, which creates a patent connection in approximately 5 to 10 days.[20,21] This gradual process induces healing with a lower inflammatory reaction when compared to stapled anastomoses.[22]

The model of magnetic loop anastomosis used in this study was easy to perform. The anastomosis healed well with minor inflammation, irrespective of the allocated group. There were no anastomotic leaks. In one case, a peri-anastomotic perforation was observed during the POD 7 endoscopic control. It is difficult to establish if the perforation was iatrogenic. However, at necropsy, there were no signs of pre-existing peritoneal inflammation. The 2 cases of bowel occlusion (both in the CD-CO2 group) were probably related to a loop malfunctioning, with the anastomosis which was not patent yet. Recently, Bai et al[23] have reported a technique of colonic resection and magnetic anastomosis in rats, demonstrating that it was faster and it healed better than using manual suturing. Our experimental model could be particularly useful to test the impact of various anastomotic healing interventions, mechanical or chemical, by reducing the bias related to the interruption of the blood supply.

The endoscopic follow-up of the animals allowed to monitor the anastomotic progressive formation process. No differences were found in terms of magnet expulsion or anastomotic completion time.

BP is a largely used surrogate metric of anastomotic healing. In a rat model, De Oliveira et al[24] demonstrated that colonic anastomosis performed under hypothermic conditions (32±1°C) led to significantly lower BP values than anastomosis performed in preserved normothermia. In our study, BP was similar in the 3 groups. It has to be underlined that none of our animals were so profoundly hypothermic and that our anastomosis model, without resection and using magnetic compression, led to a strong anastomosis in all cases. In fact, the anastomosis did not rupture under pressure, but the leak occurred in the colon wall far from the anastomotic site. Another important point is that BP was not performed in dead or sacrificed rats (mostly from the CD-CO_2 group).

In the second part of the experiment, we did not perform histology nor BP to focus on the research of potential cellular metabolism changes, upon exposure to CO_2. We used a powerful tool to quantify the metabolites profile on the anastomosis. The analyses revealed the same proportions of the metabolites in the 3 groups. The significantly lower IL-1 levels in the WH-CO_2 group at 10 days can be hardly attributed to the intraoperative insufflation in this experiment.

The most relevant data were the confirmation that WH-CO_2 allows to better preserve the core and local temperature of the exposed abdominal tissues during the surgical procedure.

The strong point of our study lies in the robustness of the design. However, it suffers from some limitations. First, the survival period was probably too long with few controls in the early postoperative phase. Six animals of the CD-CO_2 group (25%) were lost before reaching the scheduled endpoint assessments. This might have led to a significant loss of data. Second, the insufflation period was probably too short to trigger a significant response. We chose 1h duration of insufflation based on the mean time of an open colorectal procedure. Third, there was no simultaneous histological and metabolomics profiling of the anastomosis, which could have been essential to establish some correlation between the healing and the metabolic status. Fourth, the anastomotic model chosen not only allowed to eliminate any technical bias but also led to healing in almost all cases. However, it did not represent the surgical stress of a resection/anastomosis with impaired vascular supply.

Further studies are required to demonstrate the potential direct or indirect benefit of CO_2 on anastomotic healing and on the regenerating tissue metabolic profile.

Conclusions

In this experimental setting, warmed and humidified CO_2 surgical site insufflation was effective to preserve normothermia but had no impact on anastomotic healing. The new experimental model of magnetic compression loop anastomosis is easy to perform and effective. Further studies are necessary to investigate its potential to improve the healing process of the anastomotic site in colorectal surgery.

Authors' Note

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Declaration of Conflicting Interests

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Supplemental Material

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