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Laparotomy causes loss of peritoneal mesothelium prevented by humidified CO_2 insufflation, in rats

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ABSTRACT

Introduction: Avoiding tissue desiccation is a common recommendation to reduce postoperative complications following open abdominal surgery, although difficult to achieve delicately without damaging the peritoneal mesothelium. Insufflation of humidified-warm CO_2 into the abdomen during open abdominal surgery is proposed as an invisible, effortless way to prevent desiccation. We hypothesized that desiccation during open abdominal surgery would cause loss of peritoneal mesothelium that would be prevented by insufflation of humidified-warm CO_2.

Methods: Nine Wistar rats were assigned to 1 h of anesthesia only, laparotomy only, or laparotomy with insufflation of humidified-warm CO_2. Twelve hours after treatment, rats were euthanized and tissue samples were excised. Scanning electron microscopy (SEM) and light microscopy (LM) images of visceral and parietal peritoneum were scored by two independent, blinded examiners for loss of mesothelium and other indications of inflammation, including measurement of apoptosis by detection of DNA cleavage.

Results: Loss of peritoneal mesothelium was found in peritoneum exposed to laparotomy only (SEM: $P = 0.002$; LM: $P = 0.01$), and mesothelial loss was reduced by humidified-warm CO_2 (SEM: $P < 0.001$; LM $P = 0.004$). Similarly, DNA cleavage was significantly higher on the peritoneal surface following laparotomy only, compared with anesthesia only ($P = 0.0055$) and laparotomy with humidified-warm CO_2 insufflation ($P = 0.0003$).

Conclusions: In a rat model, exposing the peritoneal mesothelial to conditions that replicate minimum recommended air flow within an operating room causes inadvertent loss of mesothelium and signs of inflammation that can be prevented by insufflating humidified-warm CO_2 into the open abdominal cavity.

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Introduction

The peritoneal mesothelium plays an essential role in the prevention of postoperative complications including adhesion formation and peritoneal tumor implantation. Five percent of patients will have at least one readmission directly related to adhesions within 5 y of colorectal surgery, excluding appendectomies.[1] Over a 10-year period in the UK, an estimated 908 million Euros is spent on adhesion-related readmissions following lower abdominal surgery.[2]

Following damage to the peritoneal mesothelium, as inevitably occurs during surgical incision, the presence of neighboring mesothelial cells is essential to control the delicate balance between the deposition and breakdown of fibrin and to allow the mesothelium to heal adhesion free.[3-5] In the event of intraperitoneal tumor spillage, mesothelial cells are required to secrete free hyaluronic acid to bind to intraperitoneal tumor cells, inhibiting them from adhering and thereby metastasizing to the peritoneum.[5] In addition, it has been shown that tumor cells adhere preferentially to areas where the mesothelium is disrupted in acute *in vitro* human[6] and animal studies,[7,8] and in tissue culture investigations.[9]

One cause of inadvertent loss of peritoneal mesothelium is desiccation.[10-17] Desiccation is traditionally reduced using irrigating lavage and by placing wet packs into the abdominal cavity. However, criticism is growing against the unnecessary use of intraperitoneal lavage, as it may increase the risk of postoperative complications by disrupting the peritoneal mesothelium, and it is not effective in reducing the risk of surgical site infection.[3,5,18] Furthermore, it is likely that rubbing the peritoneum with a wet pack can also cause mesothelial damage.[11,19]

Insufflation of humidified-warm carbon dioxide (CO_2) into the abdominal cavity has been proposed as a therapy to reduce inadvertent damage to the peritoneal morphology caused by desiccation during open abdominal surgery.[20] Using an active humidification system and a specially designed gas diffuser, humidified-warm CO_2 can be diffused into the open peritoneal cavity at a low velocity while at a flow rate high enough to create a local environment with a high concentration of CO_2.[21] An invisible humidified greenhouse effect is created within the open abdominal cavity that improves tissue oxygenation[22] and reduces desiccation almost completely.[23] Clinical trials have shown that the open abdominal wound remains warmer and the risk of hypothermia is reduced during surgery,[24,25] and surgical costs are reduced.[26]

Following endoscopic surgery, reduction of desiccation by insufflating humidified-warm CO_2 has been shown to reduce mesothelial cell loss and inflammatory changes.[13,27-30] Therefore, it has been hypothesized that the use of humidified-warm CO_2 will also reduce loss of peritoneal mesothelium in open abdominal surgery.[31] Furthermore, despite the evidence that desiccation causes loss of mesothelium, there is a lack of evidence as to whether exposure of the mesothelium to the ambient operating room air ventilation during open abdominal surgery without CO_2 insufflation causes sufficient desiccation to result in mesothelial cell loss. In addition, investigations during laparoscopy suggest that loss of mesothelium will be by apoptosis,[32] will be proceeded by a change in parietal cellular morphology from a flat to relatively bugled cell,[27,29,33] and will increase the expression of the inflammatory marker COX-2 that is an important predictor of cancer progression.[14] It is also likely that the inflammation will extend to portions of the peritoneum that are not exposed to the desiccating environment and that submesothelial edema will occur.[34]

This research was designed to test two primary hypotheses. First, that exposure of the peritoneal mesothelial to normal operating room air ventilation during open abdominal surgery will cause inadvertent loss of peritoneal mesothelial cells compared with anesthesia only controls. Second, that insufflation of humidified-warm CO_2 into the open abdominal cavity will reduce the loss of peritoneal mesothelial cells compared with laparotomy without gas insufflation. Data were also collected to explore the hypotheses that laparotomy without gas insufflation, compared with both surgery with insufflation of humidified-warm CO_2 and anesthesia only controls, will cause bulging of parietal mesothelial cells, increased expression of the inflammatory marker COX-2, increased submesothelial cell thickness; and apoptosis.

Materials and methods

This study was approved by the University of Wollongong Animal Ethics Committee (AE 10-24). Nine female Wistar rats were used accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes.[35] Before surgery, the rats were housed two rats to a cage with ad libitum access to food and water. The rats were maintained in a temperature controlled environment with diurnal variation of light.

The animal experimental setup has previously been described in detail.[22]

Prophylactic pain relief was administered (subcutaneous meloxicam 1 mg/kg). Core body temperature was monitored every 5-10 min with a rectal thermometer. Insensible fluid replacement was delivered hourly at 10 mL/kg/h subcutaneously with warmed 0.9% sodium chloride, according to Australian guidelines for the promotion of well-being of animals used for scientific purposes.[36]

Rats were assigned to one of three groups.

1. Group C: anesthesia only control ($n = 2$)
2. Group LO: laparotomy only with controlled ambient air flow ($n = 4$)
3. Group LI: laparotomy with insufflation of humidified-warm CO_2 ($n = 3$)

Following commencement of mechanical ventilation, the abdomen was clipped and cleaned. In groups LO and LI, an inverted "L" shaped laparotomy incision (60-mm long midline incision, starting 10 mm caudal to the xiphoid process, and a 40 mm long incision across the left side of the abdominal wall, extending from the rostral end of the first incision). The abdominal wall was then gently reflected toward the lower left quadrant to expose the parietal peritoneum. The skin was