Opinion Paper

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Changes in the coelomic microclimate during carbon dioxide laparoscopy: morphological and functional implications

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Abstract: In this article the adverse effects of laparoscopic CO_2-pneumoperitoneum and coelomic climate change, and their potential prevention by warmed, humidified carbon dioxide insufflation are reviewed. The use of pressurized cold, dry carbon dioxide (CO_2) pneumoperitoneum causes a number of local effects on the peritoneal mesothelium, as well as systemic effects. These can be observed at a macroscopic, microscopic, cellular and metabolic level. Local effects include evaporative cooling, oxidative stress, desiccation of mesothelium, disruption of mesothelial cell junctions and glycocalyx, diminished scavenging of reactive oxygen species, decreased peritoneal blood flow, peritoneal acidosis, peritoneal hypoxia or necrosis, exposure of the basal lamina and extracellular matrix, lymphocyte infiltration, and generation of peritoneal cytokines such as IL-1, IL-6, IL-8 and TNFα. Such damage is increased by high CO_2 insufflation pressures and gas velocities and prolonged laparoscopic procedures. The resulting disruption of the glycocalyx, mesothelial cell barrier and exposure of the extracellular matrix creates a cascade of immunological and pro-inflammatory events and favours tumour cell implantation. Systemic effects include cardiopulmonary and respiratory changes, hypothermia and acidosis. Such coelomic climate change can be prevented by the use of lower insufflation pressures and preconditioned warm humidified CO_2. By achieving a more physiological temperature, pressure and humidity, the coelomic microenvironment can be better preserved during pneumoperitoneum. This has the potential clinical benefits of maintaining isothermia and perfusion, reducing postoperative pain, preventing adhesions and inhibiting cancer cell implantation in laparoscopic surgery.

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Introduction

Over 200 years ago, the German naturalist Alexander von Humboldt [1] explored various planetary ecosystems and defined the intricate relationships between humidity, temperature, barometric pressure, rainfall and climate. In analogy, the peritoneal cavity is a micro-ecosystem where factors such as humidity, pressure and temperature play a major physiological role. In this article, the detrimental effects of laparoscopic carbon-dioxide (CO_2)-pneumoperitoneum and coelomic climate change, and their potential prevention are reviewed.

Coelomic microclimate

The coelom forms the crucial interface between the parietal and visceral peritoneal surfaces. The physical, chemical and biological properties of the peritoneal cavity contribute to its controlled microclimate. It is isothermic and fully humidified, with an intra-abdominal pressure (IAP) of 0–3 mmHg [2, 3]. This provides stability for the function of the intra-abdominal viscera and maintains splanchnic blood flow, immunocompetence, temperature regulation and fluid balance. The total peritoneal surface area in an adult human (1.5–2 m^2) is similar to the skin surface area. The visceral component comprises approximately 70 % and parietal 30 % of the total peritoneal surface area [4]. The mesothelial surface of the peritoneum normally acts as a semipermeable membrane for solute and nutrient transfer and a barrier to larger molecules or foreign objects such as cancer cells. Changing the microclimate of the coelom can disrupt the normal homeostatic state of the peritoneum and abdominal viscera [2–15].

Carbon dioxide (CO_2) pneumoperitoneum

Carbon dioxide is an ideal gas for pneumoperitoneum as it is inert, soluble, easily diffusible, quickly buffered and excretable, commercially cheap and readily available. In comparison to CO_2, the relative solubility of oxygen respectively is 1/24 and nitrogen 1/46; relative diffusion of oxygen 1/20 and nitrogen 1/37. This means that CO_2 gas is readily absorbed and rapidly excreted. Thus the perioperative risks of gas embolism, retained gas, pneumothorax and potentially pain are less with CO_2 insufflation compared to air [5–7].

Standard pneumoperitoneum for laparoscopic surgery involves insufflation of cold, dry carbon dioxide gas. Carbon dioxide is contained in gas cylinders at a pressure of 57 atm as a liquid. As it passes the throttle valve on the cylinder head the CO_2 vaporizes, expands and cools as a result of the Joule-Thomson effect. When it enters the coelom via the gas insufflator, the relative humidity of introduced dry CO_2 gas is 0.0002% [5], with a temperature of 20 °C and a high velocity [2, 5, 9]. The use of cold-dry CO_2 gas causes a number of local effects on the peritoneal mesothelium, as well as systemic effects in the subject [5]. These can be observed at a macroscopic, microscopic, cellular and metabolic level [2–15].

Local effects include evaporative cooling, oxidative stress [10–12], desiccation of mesothelium, disruption of mesothelial cell junctions and glycocalyx, diminished scavenging of reactive oxygen species (ROS), decreased peritoneal blood flow, peritoneal acidosis, peritoneal hypoxia or necrosis, increased ROS production, exposure of the basal lamina and extracellular matrix (ECM), lymphocyte infiltration, and generation of prostaglandins and peritoneal cytokines such as IL-1, IL-6, IL-8 and TNFα [2, 5, 13, 14]. Factors that increase evaporation include an increase in gas flow ("wind") or surface area, or a decrease in humidity. The human-induced climate change, deforestation, water evaporation and barren land that von Humboldt first observed in Venezuela in 1800 has parallels to the damage to the coelomic microenvironment observed with cold, dry CO_2-pneumoperitoneum [1].

Jetstream-like effects of CO_2 insufflation

The cold, dry CO_2 stream from the pressurized gas insufflator has a velocity of up to 20–30 m/s [2, 3, 5, 15] and shows some similarities to the polar jetstream of the Northern hemisphere. Both have a rapid, relatively narrow, turbulent flow and contain dense, cold, dry gas. Both create vertical and horizontal wind shear forces causing turbulence, vortex formation and influencing (micro-)climate patterns [16]. Suction devices, tissue combustion plumes, gas evacuation systems, large CO_2 fluxes and high insufflation velocities during laparoscopic surgery further increase gas use, turbulence and vortices. Such vortices create convection currents, which can affect areas of the peritoneum distant from the direct path of the CO_2 gas jetstream [2]. Thus the entire visceral and parietal peritoneum can be affected, including phrenic nerve nociceptors on the subdiaphragmatic surface [5, 11]. The extent of peritoneal evaporation is related to the temperature and humidity of the insufflated CO_2 gas, but also the stability of its interaction with the peritoneal surface, and the total volume of insufflated gas [17]. When cold-dry CO_2 is used for insufflation, peritoneal climate change occurs, which can be detrimental to the patient [5].

Peritoneum – structural and functional physiology

Mesothelial cells originate from the mesoderm and are usually flattened squamous like cells with epithelial-like microvilli which project into the coelom. The microvilli are covered by a thin serous layer (60 μm) of peritoneal fluid and glycosaminoglycans, phospholipids, proteoglycans, surfactants and coagulant precursors secreted by mesothelial cells. This provides a protective, slippery glycocalyx barrier to abrasions, infections and tumour cell implantation and allows apposing serosal surfaces to slide without friction. Microvilli greatly increase the peritoneal surface area and potentially enhance this tribologic protective barrier. High molecular weight hyaluronan (HMWHA), a linear glycosaminoglycan, is a major component of the glycocalyx and is produced locally by the peritoneal mesothelial cells (PMC). It is a large hydrophilic anionic polymer with unique hygroscopic, rheologic and viscoelastic properties. Another component of the glycocalyx produced by the PMC is surfactant (phosphatidylcholine) which also contributes to lubrication and stable fluid balance. Movement of fluids and molecules across the peritoneal surface is facilitated by the microvilli and occurs via passive transport, transcellular transport, pinocytosis, plasmalemmal vesicles and stomata [8].

Mesothelial cells are connected by tight gap junctions, integrins and cadherins and anchored to the actin cytoskeleton. The visceral peritoneal mesothelium contains flat, squamous like cells with lower resting metabolic rate and tight gap junctions, as compared to the parietal peritoneum, which tends to contain cuboid cells with higher rates of metabolism. Peritoneal stomata exist that can allow egress of larger molecules, bacteria or cells, particularly on the parietal peritoneum of the diaphragm and anterior abdominal wall; and the omentum, spleen, liver and falciform ligament [8]. The mesothelial cells produce fibronectin, laminin and type I and IV collagen which comprise the basement membrane (BM). The submesothelial layer contains connective tissue macromolecules produced by fibroblasts. The ECM is made up of the BM and submesothelial layer with a reservoir of signalling molecules. These respond to ECM exposure or secreted growth factors such as fibroblast growth factor (FGF) [4, 5, 18]. The peritoneal integrins are comprised of an α (α_2, α_3, α_5, α_6, or α_v) and β (β_1 or β_3) subunit combination. They are important in attachment of cells to the ECM and cell–cell interaction on the mesothelial surface ($\alpha_2\beta_1$ and $\alpha_3\beta_1$). Intercellular protein molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) are also integrin adhesion molecules which are expressed on the microvillus surface and can interact with HA, leucocytes, endometriosis and cancer cells. Cluster of Differentiation 44 (CD44) and Hyaluronan–mediated motility receptor (RHAMM) are the two major signal transducing HA mesothelial surface receptors which influence cell–cell interaction, cell migration, cellular proliferation and inflammation. PMCs secrete matrix metalloproteases (MMP) which digest collagens and remodel the ECM. This is regulated by TGF-β and is crucial in adhesion formation and endometriosis or cancer cell invasion of the ECM [9, 14]. The PMC thus control fluid and cell transport, initiation and resolution of inflammation, leucocyte migration, tissue repair, lysis of fibrin deposits preventing adhesion formation, protection against invading microorganisms and, possibly, tumour dissemination in the coelomic cavity [8].

Evaporative cooling and the peritoneum

Dry cold CO_2 insufflation (0.0002% RH, T = 21 °C) causes rapid evaporative cooling of the peritoneum. This occurs directly from the CO_2 gas jet steam, and indirectly in the surrounding peritoneum, as the pneumoperitoneum is established and maintained. Desiccation of the peritoneal mesothelium begins within 30 s with direct gas flow and in 8–10 min with indirect gas flow [2, 5, 19]. Along the direct path of the CD CO_2 jetstream the exposed tissues can be rapidly cooled with a tissue surface temperature drop of 18 °C within 6 s over a 2 cm^2 area at gas flow rates above 5 L/min. Evaporation caused by the rapid flow of cold, dry CO_2 increases the viscosity of the glycocalyx and creates a tissue surface vapour pressure differential between the mesothelial cell and its coelomic surface [2]. This leads to shortening and loss of microvilli, disruption of the glycocalyx, bulging and rupture of mesothelial cells with exposure of the basement membrane (see Figure 1.) High CO_2 flow rates and insufflation pressures, or prolonged pneumoperitoneum times are associated with increased damage and inflammation [13]. Loss of the tribologic properties of the peritoneal fluid impairs its lubricating ability and its normal fibrinolytic and inflammatory responses. This can result in greater postoperative adhesion formation, prolonged recovery, ileus, shoulder tip/subdiaphragmatic pain and potentially tumour cell adhesiveness [2, 5, 18–20].

Pneumoperitoneum-induced hypothermia

Detrimental effects of cold-dry CO_2 insufflation during laparoscopic surgery include both systemic and intra-abdominal hypothermia [5, 19, 21]. Jacobs 1999 found that during cold-dry CO_2 laparoscopic procedures in patients, intra-abdominal temperature decreased to as low as 27.7 °C (average 32.7 °C) depending on the length of procedure (23 min–5 h 8 min), total gas volume used (12.8–801 L), CO_2 gas flow (up to 20 L/min), and leakage rate [21]. Preoperative and postoperative temperature comparisons showed no decline in rectal core temperature (average + 0.18 °C) because warming equipment (Bair Hugger®, fluid warmer, Blanketrol® blankets) was sufficient.

General anaesthesia also causes systemic hypothermia due to exposed skin, evaporation, radiated heat loss, peripheral vasodilation, cold IV fluids, anaesthetic drugs and disruption of normal thermoregulation. Heat loss from the skin of the anaesthetized patient to the operating room environment occurs due to radiation (60%), conduction and convection (15%), and evaporation (22%) [22].

Intra-abdominal hypothermia during laparoscopy is related to the energy consumed to humidify the dry CO_2 gas, (577 cal to vaporize 1 g of water), rather than the

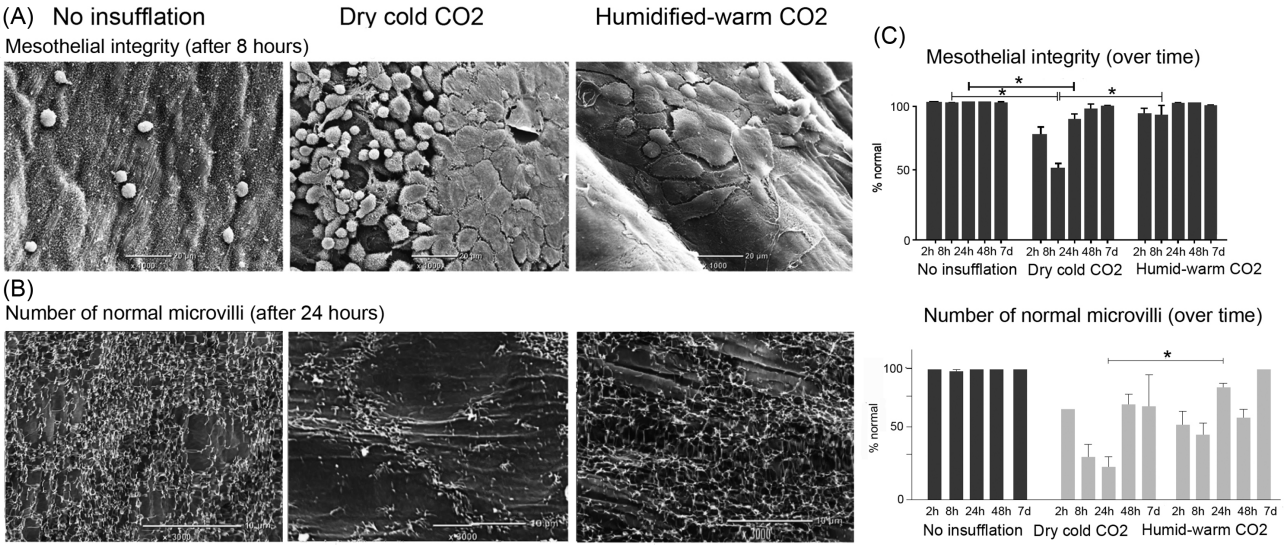

Figure 1: Changes to the mesothelium under SEM with CD CO_2 vs. WH CO_2.
(A) Representative SEM at 1,000× mag at 8 h after laparoscopy showing loss of mesothelium or retraction and rounding of mesothelial cells in CD CO_2 vs. WH CO_2, under controlled laparoscopic murine model of 2 mm IAP and 14–52 mL/min of CO_2 insufflation. (B) Representative SEM at 3,000× magnification showing loss of microvilli and exposure of ECM at 24 h in CD CO_2 compared to Control or WH CO_2. (C) Graph of percentage of normal microvilli vs. time and percentage of normal mesothelium vs. time in control vs. CD CO_2 vs. WH CO_2. Adapted from Carpinteri et al. [18].

energy required to warm the cold CO_2 to body temperature (0.00003 cal to heat 1 mL of CO_2 by 1 °C) [23]. To fully humidify a dry CO_2-pneumoperitoneum at 21 °C or 37 °C requires a similar amount of energy [24]. Thus intra-abdominal hypothermia is more related to pneumoperitoneum induced evaporation than heat transfer from warm tissues to a cold gas [2, 3, 25]. Ott (1998) estimated a net decrease in core temperature of 0.3 °C per 60 L of cold-dry CO_2 gas insufflated during pneumoperitoneum in human patients without external surface warming devices [19]. When water is vaporized it is lighter than CO_2 gas, and displaces some of the CO_2 molecules for a given volume of gas. This means cold-dry CO_2 is denser than warm humid CO_2, and larger total volumes of insufflated cold-dry CO_2 gas are required for the same procedure.

Warm, humidified CO_2 insufflation during laparoscopic surgery (e.g. Humigard®, Fisher and Paykel, Auckland, New Zealand) can prevent intraperitoneal hypothermia [5]. Matsuda (2002) in a porcine model showed a cold-dry CO_2-pneumoperitoneum after 2 h with a gas leak of 10 L/min resulted in a fall of intra-abdominal temperature to 30.1 °C and relative humidity of 88.9 % ($p<0.05$), with a non-significant fall in core temperature of −0.2 °C. In the cold-dry CO_2 group without a gas leak, intra-abdominal temperature and humidity were stabilized after 10 min (36.0 °C and 98.0 %) and remained normal; core temperature after 2 h insufflation was 36.0 °C. The core and intra-abdominal temperatures in the warm humid CO_2 with no leak vs. warm humid CO_2 with gas leakage were the same (36.1 °C); relative humidity was similar (100 % vs. 98.6 %) [17].

Use of forced air external warming

Forced air external warming devices provide both insulation from heat loss and active cutaneous warming. When used with forced air external warming blankets (e. g., Bair Hugger®), warm humid CO_2 insufflation has a synergistic effect in improving core temperature and minimizing systemic hypothermia [26]. The 2016 UK NICE clinical practice guidelines for the management of inadvertent perioperative hypothermia (CG65) concluded that below a core temperature of 36 °C, a difference between intervention and control of 0.2 °C was considered important. Above 36 °C, a difference of 0.5 °C was clinically significant. Core temperatures below 36 °C are associated with coagulopathy, platelet dysfunction, altered drug pharmacokinetics, delays in patient recovery, increased length of stay and potential for postoperative complications [27]. These include myocardial events, arrhythmias, surgical wound infections and increased blood loss and transfusion requirements [22].

The 2016 Cochrane meta-analysis of randomized controlled trials of warm humid CO_2 vs. cold-dry CO_2 in laparoscopic abdominal surgery showed a mean improvement in core temperature with warm humid CO_2 of +0.3 °C in a wide variety of laparoscopic procedures, and +0.7 °C in procedures lasting greater than 120 min ($p = 0.02$). Shorter procedures such as laparoscopic appendectomy did not show a significant difference in core temperature between gas insufflation types [28]. Patient and surgical factors involved in perioperative hypothermia include ASA grade II-V (the higher the grade, the greater the risk), advanced age, combined general and regional anaesthesia, major or intermediate surgery, cardiovascular co-morbidity or preoperative temperature below 36 °C [27].

Prevention of desiccation

Desiccation of peritoneal surfaces can be prevented by the use of warmed, humidified (>98 % RH) CO_2 gas in animal models and clinical trials. Ott (2003) introduced the concept of the 'desertification' of the peritoneum due to surface evaporation and desiccation with the use of dry CO_2 [2]. With cold-dry CO_2-pneumoperitoneum under SEM, drastic alterations of the peritoneal surface layer are seen, with microvilli loss, extreme desquamation of mesothelial cells, and obvious exposure of the basal membrane [13, 18, 29] (see Figure 1). In a fully water saturated CO_2 pneumoperitoneum (relative humidity 100 %) with a temperature similar to the surrounding tissues of 37 °C, no further evaporation from the peritoneal serous layer can occur [5].

The morphology of mesothelial cells under SEM when exposed to heated and humidified CO_2 is similar to control animals, with little inflammatory response [13, 29]. Inflammatory responses to pneumoperitoneum, as measured by IL-6 and CRP release, can be increased by as much as four times with cold/dry or warm/dry CO_2 in comparison to warm/humidified gas [2]. Inflammatory cell infiltration occurs in the visceral and parietal peritoneum 2 h after a laparoscopy with cold/dry CO_2 [30]. After 12 h, peritoneal macrophages and lymphocytes have been shown to fill the gaps in the mesothelial layer, recovering the basal lamina [5, 13, 31]. ROS release can occur from injured or activated mesothelial cells or from migrating inflammatory cells such as polymorphonuclear cell (PMNC) lysosomes. PMNCs can also secrete cytokines and growth factors such as transforming growth factor-β (TGF-β) and TNFα. Activated macrophages secrete IL-1, IL-6 and arachidonic acid metabolites [14].

Warm dry CO_2 insufflation

The desiccating effects of warm dry environment have been known for millennia: in their burial practices, the Tocharian peoples of the Xingjian Autonomous region in Western China desiccated and mummified the bodies of their dead in the hyper-arid climate of the Tarim desert basin [32]. In analogy, warm dry CO_2 insufflation causes desiccation and crenation of the peritoneal mesothelium. This is due to the thermodynamic losses from the abdominal cavity associated with humidifying the dry gas [5, 13]. Randomized controlled clinical trials have not shown warm dry gas to significantly improve postoperative pain or core temperature compared to cold-dry CO_2 [33–36]. In fact, after laparoscopic fundoplication, Wills et al. (2001) showed an increase in postoperative pain when warm dry CO_2 vs. cold-dry CO_2 was used [36].

Local and systemic effects of elevated intra-abdominal pressure

Raised IAP and reverse Trendelenburg position during laparoscopy also create local and systemic effects. Local effects include peritoneal and splanchnic hypoperfusion, peritoneal ischaemia and reperfusion injury, immunological and oxidative stress response disturbances, spread of tumour cells and bacterial translocation [2, 5, 18]. Systemic pulmonary effects include atelectasis, Alveolar arterial (Aa) O_2 mismatch and decreased pulmonary tidal volume/functional residual capacity/vital capacity and pulmonary compliance. Cardiovascular effects include decreased venous return, lower cardiac output and stroke volume, increased systemic and pulmonary vascular resistance and increased heart rate [2, 5, 37]. Raising the IAP can increase the systemic absorption of CO_2, causing hypercarbia and acidosis.

The systemic effects of laparoscopy are more pronounced in ASA III/IV vs. ASA I/II patients. Factors that contribute to such problems include initiation of CO_2-pneumoperitoneum, prolonged procedures or steep reverse Trendelenburg positioning [37, 38]. This is due to differences in homeostatic compensatory mechanisms, physiological reserve, pulmonary diffusing capacity and vascular compliance. Cardiorespiratory diseases such as emphysema, pulmonary hypertension, atherosclerosis or valvular/ischemic heart disease in comorbid or elderly patients can increase susceptibility to the physiological effects of pneumoperitoneum. Decreasing pneumoperitoneum gas

flow rates and insufflation pressures may be beneficial in high risk patients [39].

Hua (2014) in a meta-analysis of RCTs involving laparoscopic cholecystectomy demonstrated less postoperative pain and a shorter length of stay in low pressure (7–10 mmHg) vs. standard pressure (12–15 mmHg) insufflation [40]. However, a subsequent meta-analysis of a variety of laparoscopic procedures questioned the clinical benefits of low pressure pneumoperitoneum in healthy individuals [41].

Systemic effects of CO_2-pneumoperitoneum in animal models

Earlier studies of CO_2-pneumoperitoneum did not control for the systemic physiological changes that occur in murine animal models. Examples include not controlling for the cardiovascular effects of pneumoperitoneum, not warming the animal to prevent systemic hypothermia and not routinely intubating small experimental animals such as mice to regulate pulmonary minute volume, PaO_2, pH and $PaCO_2$. Using a standard human laparoscopic intraperitoneal pressure (IPP) of 15 mmHg or high CO_2 gas flow rates can contribute to decreased cardiac output, systemic hypotension, peritoneal hypoperfusion, hypercarbia, hypoxia and acidosis. All of these can confound the observed changes in the murine peritoneum.

An IPP of 6–8 mmHg in a rat (420–490 g) is comparable to an IPP of 15 mmHg in a human. However, insufflation to 8 mmHg in a 20 g mouse is not equivalent to what occurs in a human from a haemodynamic standpoint [42, 43]. Using a more proportionate insufflation IPP of 5 mmHg and CO_2 flow rate of 50 mL/min in rats and 2 mmHg and 14–52 mL/min in mice, with routine ETT intubation and pulmonary ventilation, control for these changes. This allows for more reproducible experimental conditions and reliable studies [13, 18, 42, 43]. The absorption of CO_2 is also related to excess IPP. This results in hypercarbia and systemic metabolic acidosis, leading to decreased haemoglobin O_2 affinity and decreased O_2 availability in tissues [44]. High IPP have been shown to cause peritoneal hypoxia compared to lower IPP with CO_2-pneumoperitoneum [42, 43]. During open abdominal surgery, however, intra-abdominal tissue O_2 tension was increased by local insufflation of warm humid CO_2 in a rat model. The degree of improvement in peritoneal oxygenation (PtO_2) was 28.8 mmHg or 96.6 % [45].

Local effects of CO_2-pneumoperitoneum: peritoneal hypoxia and hypoperfusion

The normal blood flow of the human peritoneum is 1–2 % of cardiac output. This is equivalent to 60–100 mL blood/minute [4, 46]. The decrease in cardiac output related to pneumoperitoneum is not sufficient to explain the decrease in splanchnic blood flow [11]. Pneumoperitoneum affects the splanchnic macro- and microcirculation, leading to compression of portal and IVC venous outflow, stasis of blood flow and visceral ischaemia [47, 48]. The resulting splanchnic arteriolar vasoconstriction is regulated by renin, angiotensin, CNS vasopressin and intrinsic splanchnic myogenic vasoconstriction [11, 49].

Schilling (1997) investigated the effect of increasing IAP pressure on splanchnic blood flow during laparoscopic appendicectomy or laparoscopic cholecystectomy in humans. Increasing the IAP from 10 to 15 mmHg decreased the blood flow to the: parietal peritoneum by 60 %, colon by 44 %, stomach by 40 %, liver by 39 %, jejunum by 32 % and the duodenum by 11 % [50]. The parietal peritoneum, fallopian tubes and stomach are the organs most susceptible to hypoperfusion when the IAP is raised from baseline to 15 mmHg [50]. Further lack of perfusion results from 14 to 20 mmHg IAP, due to the creation of intra-abdominal hypertension or abdominal compartment syndrome [47]. This leads to intestinal submucosal/mucosal and peritoneal mesothelial acidosis [51], ROS formation and hypoxia. Schilling concluded that insufflation pressures during CO_2 laparoscopic procedures (in humans) should be limited to 10 mmHg or less to avoid splanchnic microcirculatory disturbances [50].

Pneumoperitoneum-induced mesothelial oxidative stress

Increased intra-abdominal pressure and the duration of CO_2-pneumoperitoneum both induce mesothelial oxidative stress [10–12]. Mesothelial oxidative stress can be accurately measured by determining the peritoneal level of tissue 8-iso prostaglandin F2α (8-iso PGF2α). 8-iso PGF2α is a quantifiable and reliable marker of oxidative stress in humans. It is generated in cell membranes by direct free-radical species in situ reactions with arachidonic acid. It is a biologically active compound, with potent vasoconstrictor and mitogenic properties. An IAP of 5 mmHg produced relatively

little oxidative stress compared to 10 or 15 mmHg IAP in experimental animals. However, release of the insufflation was associated with a significant rise in peritoneal 8-iso PGF2α, even in the 5 mmHg IPP animals. This can be explained by an hypoxia-reperfusion injury model. Open surgery or gasless laparoscopy did not cause a rise in peritoneal 8-iso PGF2α [48].

Hypoxia inducible factor

Hypoxia inducible factor (HIF) is the master regulator of adaptive responses to tissue hypoxia, oxidative stress or injury [52]. It is a heterodimeric transcription factor comprised of hypoxia regulated HIF-α; and constitutively expressed HIF-β which is also called aryl hydrocarbon nuclear translocator (ARNT). There are 3 forms of HIF-α, including HIF-1α, HIF-2α, HIF-3α. HIF-α is targeted by 3 isoforms of HIF prolyl-4-hydroxylase (HIF PHD1, HIF PHD2, HIF PHD3) and by asparaginyl hydroxylase, also called FIH (Factor Inhibiting HIF). The HIF heterodimer directly activates hypoxia inducible transcriptional targets (the hypoxia response element) in angiogenesis (VEGFA, VEGFR-1, PAI-1), erythropoiesis (EPO), anaerobic glycolysis (GLUT-1, hexokinase-2, 6-phosphofructo-1-kinase, glyceraldehyde-3-phosphate dehydrogenase, aldolase A, enolase-1, phosphoglycerate kinase-1, lactate dehydroxygenase A, 6-phosphofructo-2-kinase), cell growth (IGF-1, Nip3), transcription (differentiated embryo chondrocyte DEC 1 and 2), cellular transport (transferrin, ceruloplasmin, multidrug resistance P-glycoprotein), and cell migration (CXCR4, c-MET) [52].

There is usually minimal HIF-α levels at normal tissue oxygen levels due to rapid metabolism of HIF-1α and HIF-2α by ubiquitin-proteasomal pathways. This is dependent on the presence of oxygen, iron and 2-oxoglutarate-dependent-dioxygenases (PHD, FIH). A reducing agent such as ascorbate is also necessary for maintaining iron in its ferrous state (Fe^{2+}) for catalytic activity of PHD. Thus PHDs have evolved as oxygen sensors as well as iron and 2-oxoglutarate sensors [53].

HIF-α degradation is regulated by the post-translational hydroxylation of conserved prolyl residues in HIF-α. The von Hippel-Lindau tumour suppressor protein (pVHL) enables binding of the prolyl hydroxylated HIF-α to a ubiquitin E3 ligase complex that catalyses ubiquitinylation of HIF-α. This targets it for hydrolysis by the ubiquitin–proteasome pathway. Hydroxylation increases the affinity of HIFα peptides for the pVHL–elonginB–elonginC (VBC) complex by at least three orders of magnitude [52].

However, if intracellular oxygen levels drop below 200 μmol/L or iron levels become limiting, HIF PHDs fail to hydroxylate the two-specific proline residues of the HIF-1*α* protein. Thus the nonhydroxylated HIF-1*α* becomes stabilized, and translocates to the nucleus. It then binds with constitutively expressed HIF-β to form HIF-1*α*/HIF-1β heterodimers [53]. Upregulation of the hypoxia response element by the HIF-1*α*/HIF-1β heterodimer then results, with rapid promotion of angiogenesis, glycolysis, peritoneal adhesions, cell motility and migration, epithelial–mesenchymal transition (EMT) and tumour growth [52, 54, 55]. This can occur during laparoscopy, particularly if high insufflation pressures are used [56].

HIF-1α can also be activated during normoxia. Prostaglandins, TGFβ, TNFα, NO and IL-4 have been shown to stimulate HIF-1α expression and transcription under normoxic conditions, and enhance amplification of HIF-1α under hypoxia [57–59]. Arachidonic acid increases hypoxia induced IL-6 production in murine embryonic stem cells via NF-κB, p38 MAPK, and HIF-1α pathways [60]. In transgenic mice deficient for hypoxia inducible factors HIF-1α and HIF-2α, postoperative adhesions related to pneumoperitoneum were abolished [61]. Trials using knockout mice for VEGFA, VEGFB or PIGF suggested mesothelial hypoxia caused intraperitoneal adhesions [62, 63]. HIF upregulates expression of VEGF by binding to the hypoxia–responsive element of the VEGF gene (see Figure 2).

Role of the glycocalyx in peritoneal integrity

The state of the glycocalyx is pivotal in cell-cell contact, tissue hydration, regulation of inflammation, tissue remodelling, and flow of nutrients and growth factors across the peritoneal membrane. The integrity of the glycocalyx is in part related to the presence of negatively charged proteoglycans and hyaluronan [64].

Hyaluronan

Hyaluronan is an important glycosaminoglycan constituent of endothelial and mesothelial glycocalyx. It protects ECM components during inflammation and prevents release of proinflammatory mediators by their sequestration in an intact scaffold of HA cross-linkage [65]. In endothelial cells it acts as a mechano-shear sensor, regulates NO release, and maintains vascular permeability [66]. HA and HA binding proteins

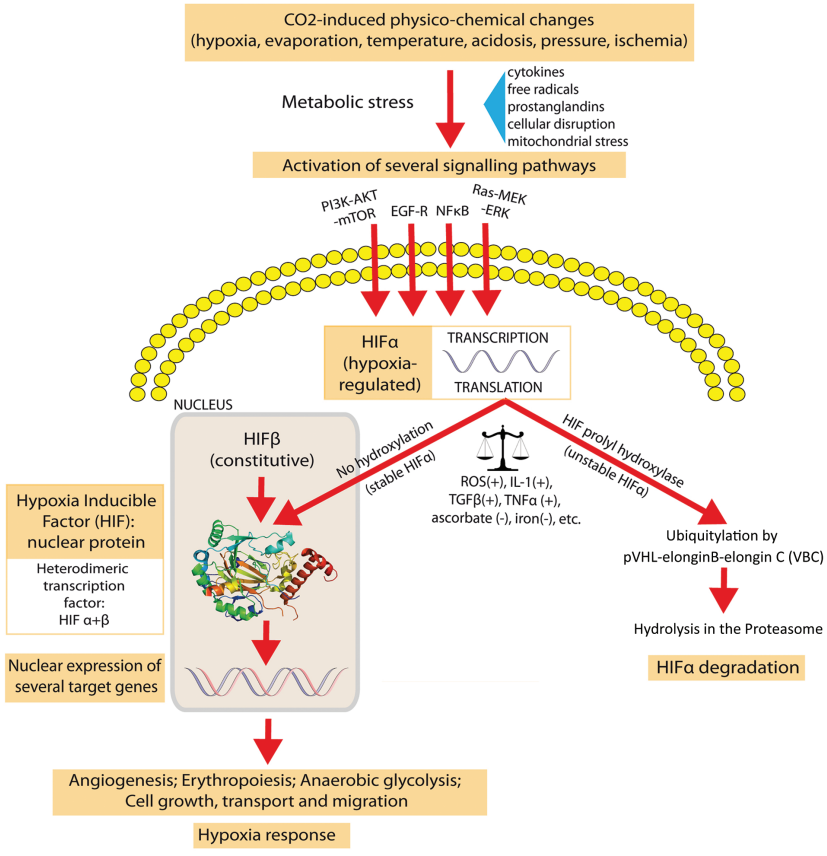

Figure 2: Physicochemical effects of CO_2-pneumoperitoneum on PMC metabolic stress and increased expression and stabilization of HIFα: mechanism of activation of the hypoxia response element and role of cytokines, ROS, PG, TNFα, TGFβ.

may enhance inflammatory responses to injury, or in other situations, may be protective of the host. This is dependent on the HA size, timing of HA deposition, and location of HA binding proteins. Production of high molecular weight (MW) HA (106 kDa) is controlled by HA synthase (HAS)- 1 and -2, and low MW HA by HA synthase-3. High MW HA inhibits inflammation and angiogenesis, while low MW HA enhances these [65, 67]. Release of disrupted HA is part of the damage associated molecular patterns (DAMPs) response to cellular injury, including hypoxia, ischaemia and mechanical stress. Low MW HA oligomers but not high MW HA induce vascular endothelial cell proliferation, enhance CD44 cleavage by tumour cells, promote tumour cell motility in a CD44/RHAMM dependent manner, increase VCAM-1, ICAM-1 and monocyte chemotactic protein (MCP-1). When mouse embryonic stem cells were exposed to mixed esters of HA with butyric and retinoic acid the expression of VEGF, VEGF receptor KDR and HGF were enhanced. Increased differentiation of embryonic stem cells into endothelial cells resulted [65]. Extracellular superoxide dismutase binds directly to high MW HA and inhibits oxidant induced fragmentation of HA [65]. Degradation of high MW HA is increased by inflammatory cytokines IL-1, IL-6, TNFα and ROS [66].

Hyaluronan fragmentation during CO_2-pneumoperitoneum

Fragmentation of hyaluronan during CO_2-pneumoperitoneum is related to insufflation pressures, peritoneal acidosis, generation of ROS, increased hyaluronidase activity and decreased HAS-1. Hyaluronidase-1 and -2 are more active in acidic environments, and with higher CO_2 insufflation pressures in human subjects (8 mm vs. 12 mmHg). Peritoneal acidosis increases with the length of the laparoscopic procedure and the pressure of CO_2 insufflation. A 12 mm vs. 8 mm dry CO_2-pneumoperitoneum in humans was associated with increased expression of E-selectin, connective tissue growth factor (CTGF), chemokine ligand 2 (CXCL-2), matrix metalloproteinase (MMP-9), and decreased expression of thrombospondin-2 (TSP-2). A lower IPP of 8 mm vs. 12 mmHg was associated with production of IL-10 and HAS-1, which are anti-inflammatory. E-selectin recruits neutrophils into the inflamed peritoneum. CTGF

activates peritoneal fibroblasts and is involved in adhesion formation. CXCL-2, also called macrophage inflammatory protein 2α (MIP2-α), is secreted by monocytes and macrophages and is chemotactic for neutrophils and haematopoietic stem cells. MMP-9 enables TGF-β activation which leads to induction of CTGF production and peritoneal fibrosis. IL-10 inhibits the inflammatory response by decreasing the production of IL-1, IL-6, IL-8 and TNFα [67]. HA fragments also induce inflammatory responses in haematopoietic progenitor cells related to cross talk between CD44 and CXCR4 signalling [65]. Thus the cascade of acute inflammatory changes in humans which begins with disruption of the glycocalyx and release of HA oligomers after laparoscopy is not only related to the nature and duration of the gas insufflation but also the insufflation pressure [67]. (see Figure 3.)

Postoperative adhesions

Postoperative adhesions occur due to increased fibrin production, thrombus formation, decreased blood flow, impaired fibrinolysis and healing by fibrosis rather than primary serosal re-epithelialization. This is mediated by peritoneal production of cytokines (TNFα, IL-1β, IL-6, IL-10), growth factors (TGF-α, TGF-β, PDGF, VEGF, EGF) the fibrin/coagulation cascade (tissue factor, PAR, tPA, uPA, PAI), and driven by the degree of peritoneal injury, desiccation and hypoxia.

Whether postoperative adhesions are decreased by laparoscopic surgery is controversial. After laparoscopic or open colonic surgery, both the human peritoneal cytokine response and the incidence of postoperative adhesions are similar. This suggests both operative approaches can be equally traumatic to the mesothelial surface [45, 49, 68, 69]. Peritoneal cytokine levels rise rapidly after peritoneal injury, starting with TNFα and quickly followed by IL-1β. IL-1β directly stimulates the release of IL-6, which peaks at 6–10 h after abdominal surgery and declines after day 2 [70]. IL-6 correlates with the severity of peritoneal damage or onset of major postoperative complications [49]. The dysregulated repair mechanisms that mediate adhesions after laparoscopic and open surgery may also promote cellular activation, such as peritoneal metastasis and endometriosis [9, 18, 49, 67–74].

In a laparoscopic mouse model [72], adding a small amount (3 %) of 0_2 to the insufflated humidified CO_2 or decreasing the body temperature to 32 °C decreased adhesions respectively by 32 % and 48 %. Adding dexamethasone or Hyalobarrier gel resulted in a combined respective reduction in adhesions of 76 % and 85 %. There was no significant additive effect of a recombinant tissue plasminogen activator (r-PA), a surfactant phospholipid, a calcium channel blocker diltiazem, a COX-2 inhibitor nimesulide or ROS scavengers superoxide dismutase and ascorbic acid. Addition of ascorbic acid actually increased adhesions. A criticism of the murine model of pneumoperitoneum used in this study is the humidified CO_2 insufflation pressures of 15 mmHg, which may have confounded the results [72].

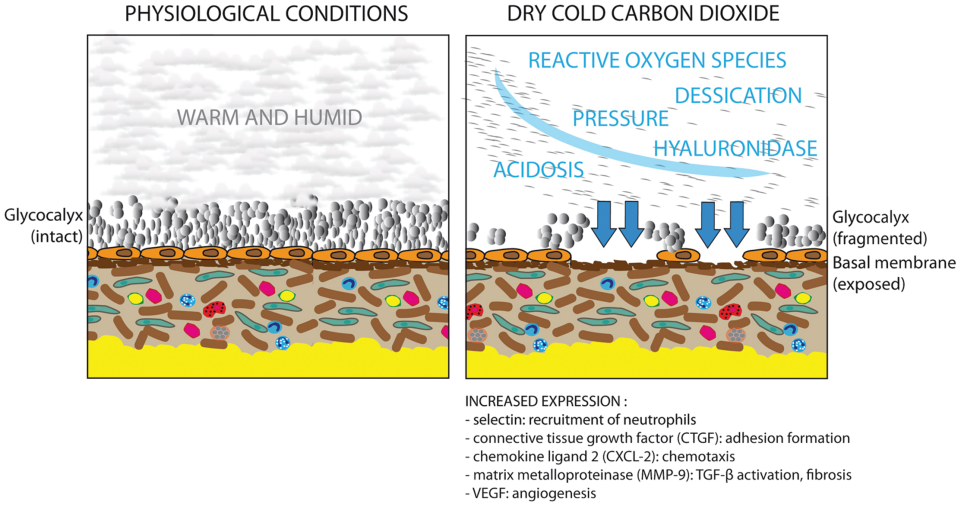

Figure 3: Denuding effects of desiccation and evaporation due to CD CO_2-pneumoperitoneum: HA fragmentation, disruption of glycocalyx, damage or loss of PMC, exposure of BM/ECM, increased cellular signalling and activation of growth factors.

Peritoneal climate change favours tumour cell implantation and growth

Peritoneal hypoxia, acidosis, desiccation and inflammation during CD CO_2-pneumoperitoneum results in mesothelial injury and cytokine production [15]. This may favour the adhesion and proliferation of epithelial malignancies such as colonic, ovarian, pancreatic and gastric carcinoma [18, 73]. Initial adhesion of tumour cells when introduced to the mesothelial surface is rapid, with binding to ECM proteins collagens type I and IV, laminin, fibronectin via integrins, and hyaluronan expressed on the surface of human peritoneal mesothelial cells via CD44 [73, 74]. Adhesion of tumour cells is increased by peritoneal injury during open surgery [71] or by cold/dry CO_2-pneumoperitoneum during laparoscopy [5, 18, 73]. Peritoneal acidosis and CO_2 insufflation results in local production of IL-1β, IL-6 and TNFα [11, 14]. Tissue adhesion molecules (ICAM, VCAM, CD44H) in human peritoneal mesothelial cells are upregulated by ROS, TNFα, IL-1β and IL-8, which can enhance the initial attachment of pancreatic carcinoma cells to the peritoneum [74, 75]. Peritoneal acidosis (pH = 6.1) during CO_2-pneumoperitoneum increases colon carcinoma cell, but not mesothelial cell, expression of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1). Both of these are important in tumour cell migration and metastatic progression [76]. VEGFA expression has been shown to be upregulated independently by hypoxia and acidosis [5, 77]. Hypoxia may stimulate EMT due to increases in TGFβ and TGFβR expression, leading to increased cancer cell migration and peritoneal metastasis. This autocrine TGF/TGFβ signalling under hypoxia was associated with an aggressive gastric carcinoma phenotype [78]. Hypoxia, TGFβ, EGF, acidic pH, and ECM-induced integrin activation have been shown to trigger invadopodia formation and cell invasion [79]. TGFβ1 is highly upregulated in gastric cancer cell lines under hypoxia, leading to induction of T regulatory cells (Treg). Treg-mediated immunosuppression allows immune evasion by tumour cells as they are able to overcome the activity of natural killer cells, dendritic cells and CD8 cytotoxic cells. Expression of HIF-1α and Treg cells were also positively correlated, but HIF-1α was not correlated with the induction of TGFβ under hypoxia [80].

IL-1β induction of inflammation, cancer cell proliferation, angiogenesis

IL-1β promotes the adherence of colorectal cancer, ovarian cancer and melanoma cells to endothelial cells in a dose-dependent way. Watanabe (2012) demonstrated that the behaviour of pro-metastatic (MFOC3) vs. non metastatic ovarian cancer cells (FOC3) were related to upregulated genomic expression of cell adhesion molecules (ADAM9, ITGA8, CTNNβ1 (Catenin, β1), fibrinolytic mediators (PAI2, uPA), cytokines (IL-1β,TNF), apoptosis inhibitors (API -1 and -2), growth factors (TGFβ1, NRG, FGF-1), ITGA (integrin α), LAMβ1 (Laminin, β1) and SDC1 (Syndecan 1) [73]. IL-1β expression was increased by 11.1 fold and PAI2 by 25.6 fold in the pro-metastatic ovarian cells compared to the non-metastasizing variant. The addition of exogenous recombinant IL-1β to mesothelial cells transformed the ability of FOC3 cells to adhere to the MCs to a similar level as the aggressive ovarian cancer cell line. There was no significant effect on mesothelial expression of α-1, 2, 5, 6, and V integrins or CD44. Endogenous IL-1β released from MFOC3 ovarian cancer cells conditioned the peritoneal mesothelial cells to express cell surface β1 integrins. This paracrine IL-1β/β1 integrin axis enabled cancer cell adhesion and proliferation and was inhibited by anti-β1 integrin antibodies. Patients with ovarian cancers expressing IL-1β had a 29 % death rate over 5 years vs. 1.6 % of patients whose tumours lacked IL-1β expression. Incubating colon carcinoma cells with factors released after surgical trauma (EGF and IL-1β) led to a 60 % greater cell adhesion to rat mesothelial cell monolayers [71, 81]. This effect can be blocked by anti-IL-1β antibodies in a dose-dependent manner. For example, 10 μg/mL of IL-1β antibody decreased MFOC3 cellular adhesion to mesothelial cells by 65 % [73].

Interleukin-1β and VEGF

IL-1β can also stimulate release of stored VEGF from the exposed peritoneal ECM, which results in endothelial cell proliferation [73]. Thus IL-1β is an upstream regulator of VEGF-induced angiogenesis. IL-1β can be secreted by damaged or activated peritoneal mesothelium, or by the tumour cell itself. IL-1β can disrupt the integrity of

human peritoneal mesothelium which leads to cell retraction and exposure of submesothelial ECM. This means that cancer cells can implant in an uninjured peritoneum when they secrete their own IL-1β, or in an accelerated way in an activated peritoneum which is expressing growth factors, cytokines, prostaglandins and adhesion molecules [71, 81]. The downstream effects of VEGF are related particularly to its activation of VEGF receptor 2 tyrosine kinase (VEGFR2), leading to a cascade of activation of c-Src, FAK, Erk 1/2, Akt, mTOR in human endothelial cells. Various human cancer cells including ovarian cancers can also produce their own VEGF and express VEGFR, leading to an autocrine stimulatory effect on tumour cell growth [82, 83]. VEGF is secreted by Cancer Associated Fibroblasts (CAF) derived from PMC which have undergone mesothelial to mesenchymal transition (MMT). This appears to determine progression and volume of malignant ascites, including ovarian and gastric carcinoma [74].

Cancer cell adherence and MMT

The ability of cancer cells to initially adhere to the peritoneal mesothelium and subsequently proliferate is determined by the 'cross talk' between cancer cells and mesothelium. This is mediated by ligand–receptor pairs, including α5β1 integrins–fibronectin, αVβ3 integrins–vitronectin (VN), and CD44–hyaluronic acid [73, 74, 84, 85]. Laparoscopic port site metastases after CO_2-pneumoperitoneum at 4–6 mmHg in BALB/c mice inoculated with human gastric carcinoma cells were diminished by addition of hyaluronic acid, anti-integrin antibodies and anti-CD44 antibody [86]. Using a CO_2 IPP of 15 mmHg compared to 9 mm CO_2 IPP or room air controls resulted in increased tumour expression of adhesion molecules CD44v6 and ICAM-1 in an *in vitro* human gastric cancer cell model [87].

PMC can undergo MMT by damage, e. g. peritoneal dialysis/CD CO_2 laparoscopy or by active recruitment by cancer cells into transformed CAF. Cancer cells themselves can also be transformed into more invasive phenotypes by EMT, which in turn promotes further PMC MMT [78, 87].

When damaged or transformed, PMC undergo MMT, which is similar to EMT, typified by loss of epithelial-like phenotype and acquisition of mesenchymal properties [74, 78]. These include loss of E-cadherin and expression of N-cadherin, loss of tight junctions, expression of mesenchymal markers: fibronectin, vimentin, fibroblast specific protein-1(FSP-1), smooth muscle actin (α-SMA) with associated increased cellular polarity and migration [9]. MMT and EMT are induced by HIF, TGF-β and repression of E-cadherin. E-cadherin transcriptional repressors include the zinc finger proteins Snail and Slug, ZEB1 (Zinc Finger E-box 1) and TWIST1, SIP1, FOXC1, FOXC2. Other cytokines and growth factors involved in MMT/EMT include hepatocyte growth factor, platelet derived growth factor (PDGF) and IL-1β [9, 14, 61–63, 84–92]. The majority of cytokines and chemokines released into the coelom during inflammation are secreted by peritoneal macrophages, with contributions from mesothelial cells, PMNC, fibroblasts or cancer cells when present [11, 19].

Prevention of tumour implantation by warm humidified CO_2

Recently Carpinteri et al. [18] showed in a controlled murine model that warmed humidified CO_2-pneumoperitoneum was associated with lower production of peritoneal VEGFA and COX-2 and decreased peritoneal colonic carcinoma cell adhesion. The small animal pneumoperitoneum model included a proportionate CO_2 insufflation pressure of 2 mmHg and CO_2 flow rate of 14–52 mL/min. Better preserved mesothelial microvilli and morphology under SEM was demonstrated in the warm humid CO_2 group. In the cold-dry CO_2 group, there was significantly more rounding and retraction of mesothelial cells (at 8 hours), and shortening or absence of mesothelial microvilli (at 24 hours) (see Figure 1.) This 'cobblestone' appearance of mesothelial cells was used as an early measure of delamination of the mesothelium from the basement membrane and cellular damage. Macrophage infiltration was significantly increased at 48 hours in the peritoneum exposed to cold-dry CO_2. It was concluded that humidified warmed CO_2 preserved mesothelial architecture and diminished peritoneal inflammation, which prevented colon carcinoma cell adhesion (see Figure 4.) Established tumour cell progression after initial implantation was not influenced by the type of CO_2 gas used. Further studies were proposed using agents such as COX-2 inhibitors to minimize peritoneal inflammation and angiogenesis during or after laparoscopy, particularly with cold-dry CO_2-pneumoperitoneum [18].

Whether the conditioning effect of WH CO_2-pneumoperitoneum in laboratory studies translates into improved

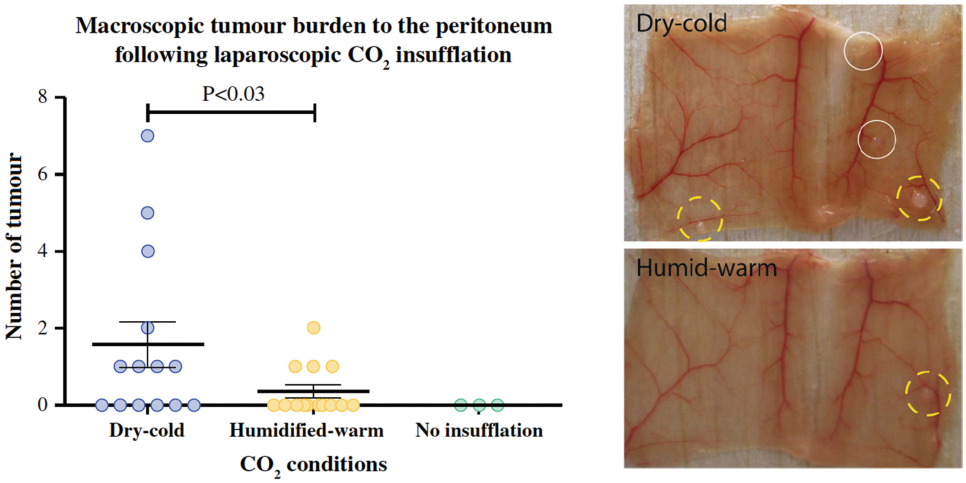

Figure 4: Implantation of colonic carcinoma (CT26) cells in mouse peritoneum 10 days after tumour inoculation and laparoscopy with cold-dry CO_2 vs. warm humidified CO_2 vs. control (no laparoscopy).
A significantly higher ($p<0.03$) tumour burden was found in mice exposed to CD CO_2 vs. WH CO_2. From Carpinteri et al. [18].

clinical outcomes in laparoscopic oncology surgery is still being debated [93]. Much of the earlier concerns about cancer cell aerosolization, increased port site metastases or decreased cancer specific survival after elective laparoscopic colorectal cancer resection in humans have been allayed by subsequent large RCTs comparing open vs. laparoscopic procedures [93]. Port site metastases are now thought to be more related to intraoperative handling of the cancer, contamination by the operating surgeon's laparoscopic instruments or increased peritoneal trauma. Laparoscopic surgery in patients with locally advanced gastrointestinal cancers where there is greater risk of tumour manipulation and dissemination is still of concern [89].

Prevention of initial tumour implantation by warmed humidified CO_2 vs. cold-dry CO_2-pneumoperitoneum may be related to preservation of the peritoneal mesothelial glycocalyx, microvilli and high molecular weight HA [13, 18, 29, 91]. Reducing peritoneal desiccation, peritoneal inflammation [5, 69] and cytokine generation can also potentially inhibit cancer cell interaction with peritoneal mesothelial cells and the ECM [18, 90, 91]. This, however, does not completely prevent tumour cell adherence and proliferation [18]. Changes in the peritoneal mesothelial microenvironment and resulting milieu after laparoscopy are capable of increasing the invasive potential of shed cancer cells via EMT and MMT [9, 70, 71, 74, 75, 78, 81]. Epithelial-mesenchymal transition (EMT) related to hypoxia, HIF-1α release, acidosis and CO_2 insufflation may still occur despite WH CO_2 conditioning or use of lower pneumoperitoneum IAP [18, 48, 50, 78]. Stabilization of HIF-1α and activation of the hypoxia response element can proceed under normoxic conditions if there is sufficient release of inflammatory cytokines or ROS to inhibit HIF-α PHD [52, 88, 92]. The contribution of high vs. low pressure pneumoperitoneum in modifying perioperative complications, adhesion formation, postoperative pain, anastomotic healing, tumour metastasis, and venous thromboembolism in human subjects remains to be established [40–43, 67, 94]. To enhance the protective effects of humidified pneumoperitoneum in the prevention of transcoelomic metastasis, additional interventions must be capable of protecting peritoneal mesothelial cells and the peritoneal glycocalyx, decreasing free radical, cytokine and growth factor production, inhibiting adhesion, attachment and activation of cancer cells, preventing peritoneal mesothelial cell senescence/MMT and macrophage immunotolerance [5, 18, 72, 80, 85, 88, 90, 91].

Conclusions

'Minimally invasive' surgery creates a wound to the entire peritoneum which may not be as minimal as the term infers [68, 95]. Use of pressurized cold, dry CO_2-pneumoperitoneum causes visceral and peritoneal hypoperfusion, rapid evaporative cooling, 'desertification' and damage of the peritoneal surface during laparoscopy. Such damage is increased by high CO_2 insufflation pressures, rapid gas velocities and prolonged laparoscopic procedures. The resulting disruption of the glycocalyx, mesothelial cell barrier and exposure of the ECM creates a cascade of immunological

and pro-inflammatory events. Such coelomic climate change can be prevented by the use of lower insufflation pressures and preconditioned warm humid CO_2 gas. By achieving a more physiological temperature, pressure and humidity during pneumoperitoneum, the coelomic microenvironment can be better preserved. This has the potential clinical benefits of maintaining isothermia and perfusion, preventing adhesions, reducing postoperative pain, and inhibiting cancer cell implantation in laparoscopic surgery.

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