F, V & V in ObGyn, 2010, 2 (3): 149-160

Review

Adhesion formation after laparoscopic surgery: what do we know about the role of the peritoneal environment?

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Abstract

In spite of the approaches that have been proposed to reduce postoperative peritoneal adhesions, they remain a major clinical problem because of the associated intestinal obstruction, chronic pelvic pain, female infertility and difficulties at the time of reoperation. The pathogenesis of the process have been focused almost exclusively on the local events induced by the surgical trauma, and the strategies for adhesion prevention thus focused on barriers to separate surgically denuded areas. The important role of the peritoneal cavity environment only recently became apparent and is not yet incorporated in adhesion reducing strategies. Recent data demonstrate that, in the presence of a direct surgical trauma, the entire peritoneal environment is quantitatively the most important factor in adhesion formation and hence adhesion prevention after both open and laparoscopic surgery. Indeed mesothelial hypoxia (CO_2 pneumoperitoneum) or hyperoxia (open surgery), desiccation and surgical manipulation have been identified as factors cumulatively enhancing adhesions. The clinical implication is especially relevant for laparoscopic surgery because the pneumoperitoneum, being a closed environment, can be easily conditioned. Although human studies are lacking, animal data indicate that peritoneal adhesions can be reduced by over 80% with a good surgical technique, with adequate pneumoperitoneum conditioning as adding 3-4% of oxygen to the CO_2 pneumoperitoneum, prevention of desiccation and slight cooling. Adhesion prevention barriers remain additionally effective, although quantitatively less important. The relevance of all these strategies for adhesion prevention still have to be confirmed in humans, but since it seems that the peritoneal environment is quantitatively much more important than the surgical trauma, adhesion prevention research and strategies should be directed more to conditioning the peritoneal cavity than to the use of agents.

Key words: laparoscopy, pneumoperitoneum, adhesion formation, adhesion prevention.

Aetiology and types of adhesions

Adhesions are pathological fibrous connections between surfaces within body cavities (e.g. peritoneal, pericardial, pleural, uterine, joint cavities). Peritoneal adhesions can be congenital or acquired, which can be postinflammatory or postoperative (Ellis, 1997). Abdominal surgery is the most common cause of adhesions, 70-85% of all adhesions being attributed to previous surgery (Weibel and Majno, 1973). On the other hand, surgery has been documented as causing adhesions in 55% to 100% of the cases (Diamond, 2000).

Different types of adhesion formation can be distinguished (Diamond and Nezhat, 1993):

Type 1 (*de novo* adhesion formation): adhesions formed at sites that did not have adhesions previously.

Type 1A: no previous operative procedures at the site of adhesions.

Type 1B: previous operative procedures at the site of adhesions.

Type 2 (adhesion reformation): adhesions formed at sites where adhesiolysis was performed.

Type 2A: no operative procedures at the site of adhesions besides adhesiolysis.

Type 2B: other operative procedures at the site of adhesions besides adhesiolysis.

Clinical significance of adhesions

Depending on their structure and location, peritoneal adhesions may remain silent or cause clinically important complications, such as intestinal obstruction, female infertility, chronic pelvic pain and difficulties at the time of reoperation.

Intestinal obstruction is the most serious complication of peritoneal adhesions as it can be life threatening due to strangulation. Adhesions are the leading cause of intestinal obstruction, accounting for more than 40% of all cases of intestinal obstruction and for 60-70% of those involving the small bowel (Ellis, 1997).

Chronic pelvic pain has been associated with adhesion formation. Although it has been reported that adhesions cause chronic pelvic pain in some 25% of patients (Steege, 2000), from a clinical point of view this association is unclear because does not necessarily imply a causal relationship. Indeed, it was demonstrated that a large number of infertility patients with adhesions do not experience pelvic pain (Steege, 2000). It was suggested that pelvic pain is a consequence of the restricted organ mobility imposed by adhesions and a relief of symptoms after adhesiolysis was reported (Steege, 2000).

Female infertility also has been associated with peritoneal adhesions, which are well recognised as a cause of distortion of the peritoneal factor due to the restrictions of the sweeping of the fimbria over the ovary. Periadnexal adhesions were found in some 20-30% of infertile women and marked increases in pregnancy rates were reported after adhesiolysis (Marana and Muzii, 2000).

Adhesions increases the technical difficulties for repeated surgeries (e.g., access to the abdomen and/or to the operative site, complication rates, anaesthesia, operating and recovery time, use of surgical materials and need for blood transfusion). Therefore, the magnitude of adhesions related disorders (ARD) is larger than could be anticipated and is better illustrated by the reports showing that hospital readmission for ARD rival the number of hip replacements, heart bypass or appendix surgeries, that 35% of women having open gynaecologic surgery are readmitted 1.9 times in 10 years for operation due to adhesions or complicated by adhesions, and that the estimated annual cost for ARD in the USA is 1.3 billion US$ (Ray *et al.*, 1998).

Pathogenesis of peritoneal adhesions

The peritoneum, with a surface are of some 10000 cm^2 in adults, almost equal to that of the skin, is the largest organ in humans (diZerega, 1997). It serves to minimise friction and facilitate free movement of abdominal viscera, to resist and localise infections and to store fat. It is composed of a continuous layer of mesothelial cells and a layer of loose connective tissue (diZerega, 2000). Mesothelial cells are highly differentiated in the peritoneum, as well as in the pleura and the pericardium, and their apical surface contain abundant long microvilli that increase the functional surface for absorption and secretion. Mesothelial cells secrete glycosaminoglycans, proteoglycans, and phospholipids to provide a slippery, non-adhesive glycocalyx that protects the serosal surface from abrasion, infection, and tumour dissemination. In addition, mesothelial cells can synthesize cytokines, chemokines, growth factors, and matrix components that regulate inflammation; initiate cell proliferation, differentiation, and migration; and mediate tissue repair (Yung and Chan, 2007). Mesothelial cells are connected to one another by desmosomes and very loosely attached to the underlining basement membrane. The connective tissue is composed of bundles of collagenous and elastic fibres oriented in different directions and a rich network of blood and lymphatic vessels. Interspersed among these fibres and vessels there are poorly differentiated epithelioid-like cells, fibroblasts, macrophages, mast cells and fat cells (diZerega, 2000).

The intact peritoneal cavity contains 3-50 ml of peritoneal fluid with plasma proteins, including a large amount of fibrinogen, and a variety of free-floating cells, including macrophages, lymphocytes, eosinophils, mast cells and desquamated mesothelial cells (diZerega, 2000).

Peritoneal injury, due to surgery, infection or irritation, initiates an inflammatory reaction that increases peritoneal fluid's proteins and cells, generating a fibrinous exudate and fibrin formation (Holmdahl, 1997). This is the result of the activation of the coagulation cascade, which includes two pathways (i.e., the contact factor or intrinsic pathway and the tissue factor or extrinsic pathway). Activation of these pathways transforms prothrombin (Factor II) into thrombin (Factor IIa) via the common pathway. Thrombin then triggers the conversion of fibrinogen into monomers of fibrin, which interact with each other and polymerise. The initially soluble polymer becomes insoluble by some coagulation factors such as Factor XIIIa and is deposited on the wound surface (Holmdahl, 1997). Within this fibrinous exudate, polymorphonuclears (PMN), macrophages, fibroblasts and mesothelial cells migrate, proliferate and/or differentiate. During the first two postoperative days, a large number of PMN enter and, in the absence of infection, depart within 3-4 days.

Macrophages increase in number and change their functions, becoming the most important component of the leukocyte population after day 5. They phagocyte more accurately, have greater respiratory burst activity and secrete a variety of substances including cytokines and growth factors that recruit new mesothelial cells onto the injury surface. Mesothelial cells migrate, form islands throughout the injured area and proliferate in order to cover the denuded area. This reepithelialisation process is different from that occurring in the skin because the entire surface becomes epithelialised simultaneously from the islands of mesothelial cells and not gradually from the borders. Therefore, it is irrespective of the size of the injury and is complete in 5-7 days (diZerega, 2000). The mechanism of mesothelial healing suggests the involvement of stem cells in the process, which is consistent with the fact that mesothelial stem cells can differentiate into mesothelial cells and a few other phenotypes and that mesothelial cells are themselves stem cells (Lucas, 2007).

PMN, macrophages, fibroblasts and mesothelial cells release a variety of substances including plasminogen system components, arachidonic acid metabolites, reactive oxygen species (ROS), cytokines and growth factors, which modulate the process of peritoneal healing and adhesion formation at different stages (Chegini, 2008, Holmdahl, 1997).

Although the fibrinous exudate and fibrin deposition are essential parts of normal tissue repair, a complete resolution is required to restore the preoperative peritoneal conditions. The degradation of fibrin is regulated by the plasminogen system, in which the inactive proenzyme plasminogen is converted into active plasmin by plasminogen activators (PAs), a process that is inhibited by plasminogen activator inhibitors (PAIs) (Holmdahl *et al.*, 1997). Plasminogen is a glycoprotein synthesised in the liver that is abundant in almost all tissues. It is the inactive precursor of plasmin, a serine protease that is highly effective in the degradation of fibrin into fibrin degradation products (FDP) and that has a role in other stages of tissue repair, such as extracellular matrix (ECM) degradation (Wong *et al.*, 1992), activation of proenzymes of the matrix metalloprotease (MMP) family (Murphy *et al.*, 1992), and activation of growth factors (Saksela and Rifkin, 1990). The principal activator of plasminogen is the serine protease tissue-type PA (tPA), which is expressed in endothelial cells, mesothelial cells and macrophages. tPA has a high affinity for fibrin and binds to a specific receptor, which exposes a strong plasminogen-binding site on the surface of the fibrin molecule. Therefore, in the presence of fibrin the activation rate of plasminogen is strikingly enhanced, whereas in the absence of fibrin tPA is a poor activator of plasminogen (Ichinose *et al.*, 1986; Norrman *et al.*, 1985). This results in higher plasminogen activation on the sites where it is required, whereas systemic activation is prevented. The other activator of plasminogen is the serine protease urokinase-type PA (uPA). The properties of uPA differ from those of tPA as it lacks high-affinity binding for fibrin and thus the increased activity in the presence of fibrin. Therefore, uPA is limited in its capacity to activate plasminogen (Lu *et al.*, 1992).

PAs can be counteracted by PAI-1 and PAI-2 through the formation of inactive complexes. The glycoprotein PAI-1 is the most potent inhibitor of tPA and uPA and is expressed in endothelial cells, mesothelial cells, macrophages, platelets and fibroblasts. The glycoprotein PAI-2 is a poorer inhibitor of tPA and uPA and is expressed in mesothelial cells, macrophages and epithelial cells. Other two PAIs have been identified (i.e., PAI-3 and protease nexin 1), but their roles in peritoneal fibrinolysis remain unknown. Plasmin can be directly inhibited by plasmin inhibitors (i.e., 2-macroglobulin, α2-antiplasmin and α1-antitrypsin), but their roles in peritoneal fibrinolysis are not well defined either (Holmdahl *et al.*, 1997).

The balance between fibrin deposition and degradation is critical in determining normal peritoneal healing or adhesion formation. If fibrin is completely degraded, normal peritoneal healing will occur. In contrast, if fibrin is not completely degraded, it will serve as a scaffold for fibroblasts and capillary ingrowth. Indeed, fibroblast will invade the fibrin matrix and

ECM will be produced and deposited. The ECM can be completely degraded by MMPs, leading to normal healing. However, if this process is inhibited by tissue inhibitors of MMPs (TIMPs), peritoneal adhesions will be formed.

Laparoscopic surgery and adhesion formation

It has been claimed that laparoscopy is less adhesiogenic than laparotomy but the data are not conclusive. Some authors reported fewer type 1B adhesions after laparoscopy than after laparotomy in rats (Schafer *et al.*, 1998), dogs (Schippers *et al.*, 1998), pigs (Garrard *et al.*, 1999) and rabbits (Luciano *et al.*, 1989), whereas others failed to show differences in rats (Filmar *et al.*, 1987) and in rabbits (Jorgensen *et al.*, 1995, Marana *et al.*, 1994). It was also reported fewer type 1A and type 2A-B adhesions after laparoscopy in rabbits (Luciano *et al.*, 1989), which was not confirmed in other studies in rabbits (Marana *et al.*, 1994). In humans, the only

RCT comparing laparotomy and laparoscopy (i.e., patients who underwent surgical treatment for ectopic pregnancy and who then underwent a second look laparoscopy) demonstrated fewer type 1A and type 2A-B adhesions in the laparoscopy group (Lundorff, 1993). Other non-randomised clinical trial also demonstrated less adhesion formation after laparoscopy (Bulletti *et al.*, 1996; Levrant *et al.*, 1997), whereas others reported a low incidence of type 1B adhesions, a very low incidence of type 1A adhesions, and a high incidence of type 2 A-B adhesions after laparoscopy (Diamond *et al.*, 1987).

From all these animal and human data, no definitive conclusion can be drawn, but they strongly suggest that laparoscopy very seldom induces type 1A adhesions, that laparoscopy has some advantages for type 1B adhesions, and that laparoscopy is similar to laparotomy for type 2A-B adhesions.

To interpret these data it is important to highlight the differences between laparoscopy and laparotomy in terms of the direct trauma induced by the surgery itself and the indirect trauma that might be induced by the peritoneal environment (Fig. 1). If performed adequately by well-trained surgeons, laparoscopy should induce less direct surgical trauma because of gentle tissue handling, meticulous haemostasis, constant irrigation, the use of microsurgical instruments and the smaller operative field, which may reduce the risk of adhesion formation. On the other hand, laparoscopy and laparotomy are performed in different gas environments, CO_2 pneumoperitoneum for the former and air for the latter. Indeed, during laparoscopy pneumoperitoneum is necessary and CO_2 is generally used for safety reasons (i.e., its high solubility in water and its high exchange capacity in lungs). CO_2 pneumoperitoneum, however, induces some adverse systemic and local effects (Fig. 1).

CO_2 pneumoperitoneum's systemic and local effects

Systemically, CO_2 pneumoperitoneum impairs venous return depending on the intraabdominal pressure (Horvath et al., 1998) and induces CO_2 absorption from the abdominal cavity, causing acidosis and hypercarbia (Junghans *et al.*, 1997; Liem *et al.*, 1996; Volz et *al.*, 1996), which if not compensated adequately by ventilation can negatively affects the cardiovascular and respiratory function (Neuberger *et al.*, 1996; Volz *et al.*, 1996). CO_2 pneumoperitoneum also induces hypothermia (Hazebroek *et al.*, 2002b; Ott, 1991a, 1991b) and decreases splanchnic perfusion with resulting oxidative stress (Sammour *et al.*, 2009), and it is associated with postoperative pain (Helvacioglu and Weis, 1992).

Locally, CO_2 pneumoperitoneum induces desiccation (Gray *et al.*, 1999) and peritoneal acidosis (Volz et al., 1996), which may mediate suppression of peritoneal macrophage function (Neuhaus and Watson, 2004). In addition, CO_2 alters the peritoneal microcirculation decreasing the ROS scavengers (Taskin *et al.*, 1998), alters the peritoneal fluid (Ott, 2001), modulates the local immune system and the inflammatory reaction (Brokelman *et al.*, 2010), and inhibits the peritoneal plasmin system, leading to peritoneal hypofibrinolysis. Furthermore, it has been demonstrated that the pneumoperitoneum is a cofactor in postoperative adhesion formation (Fig. 1).

CO_2 pneumoperitoneum is a cofactor in adhesion formation

In an early study of our group evaluating the effect of training upon adhesion formation in rabbits it become accidentally evident that longer laparoscopic surgeries were associated with more adhesions, and that both duration of surgery and adhesion formation decreased with training (Ordonez *et al.*, 1997). Based on these observations it was assumed that the surgical trauma was more severe during the longer surgeries reported at the beginning of the study, and remained undetermined the specific contribution of each factor (i.e., surgical trauma and duration of surgery/pneumoperitoneum) to adhesion formation.

To evaluate the effect of duration of surgery, and more specifically of the pneumoperitoneum, laparoscopic rabbit and mouse models were developed and surgeries were performed by well trained surgeons in order ascertain standardised lesions to induce adhesions. The first variable evaluated was the duration of the CO_2 pneumoperitoneum. Indeed, the lesions were performed in some 3-5 minutes but the pneumoperitoneum was maintained for different time periods. Since adhesion formation clearly increased with the duration of the pneumoperitoneum the concept that pneumoperitoneum is a cofactor in adhesion formation was born (Molinas *et al.*, 2001; Molinas and Koninckx, 2000; Yesildaglar *et al.*, 1999). Subsequently, a series of experiments were carried out to assess a variety of pneumoperitoneum-related parameters and thus to elucidate the potential mechanisms involved (e.g., peritoneal hypoxia, peritoneal acidosis/hypercarbia, desiccation, hypothermia, etc.).

Pneumoperitoneum-induced hypoxia as a driving mechanism

It was hypothesised that the pneumoperitoneum compresses the capillary flow in the superficial peri-

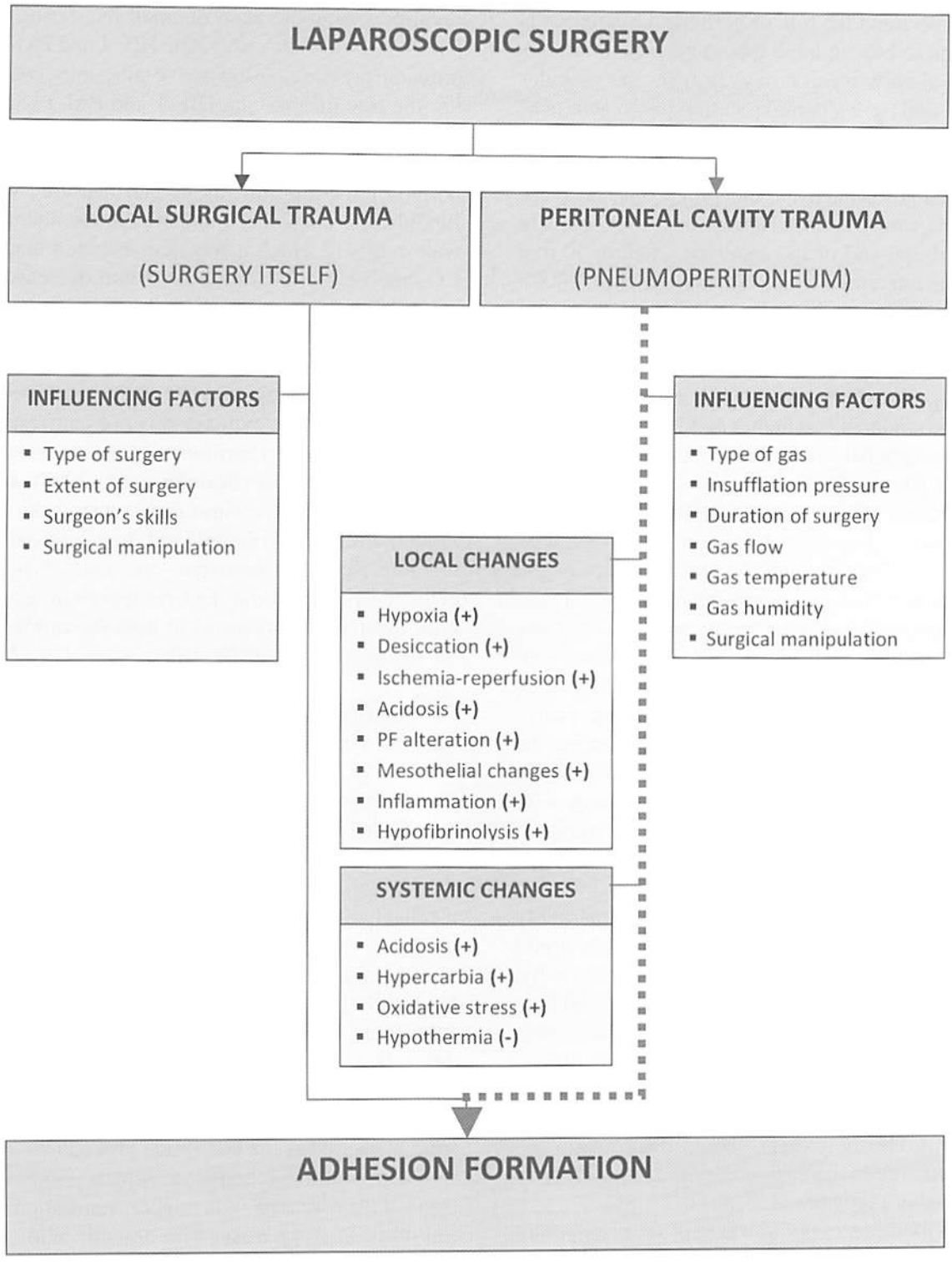

Fig. 1. — Factors involved in adhesion formation after laparoscopic surgery

toneal layers inducing ischemia, which might trigger a hypoxic peritoneal response eventually leading to adhesion formation. This hypothesis was tested by evaluating the effect of duration of pneumoperitoneum, insufflation pressure and insufflation gas.

In a series of rabbit and mouse studies using CO_2 and helium pneumoperitoneum it was demonstrated that adhesion formation increases with the duration of the pneumoperitoneum and with the insufflation pressure, without differences between both insufflation gases. Furthermore, it was reported that adhesion formation decreases with the addition of 2-4% of oxygen to the pneumoperitoneum, an effect that was no longer observed with higher oxygen concentrations (e.g., 12%) (Elkelani *et al.*, 2004; Molinas *et al.*, 2001; Molinas and Koninckx, 2000; Yesildaglar *et al.*, 1999; Yesildaglar and Koninckx, 2000).

To understand the role of peritoneal hypoxia it is important to bear in mind that in normal conditions peripheral cells receive oxygen from the vascular network and have a partial pressure of oxygen (pO_2) of 23 mm Hg (5-40 mm Hg depending on the cells type and on the distance to the capillaries). This intracellular pO_2 is the result of a progressive decrease of the pO_2 from 160 mm Hg in the air to 95 mm Hg in the arterial end of the capillaries, and to 40 mm Hg in the interstitial fluid (Guyton and Hall, 2000). The pneumoperitoneum, depending on the pressure and on the time of exposure, and independently on the insufflation gas, will compress the capillary flow in the superficial peritoneal layers, decreasing tissue perfusion, inducing ischemia and reducing the pO_2 in the mesothelial cells up to hypoxic levels (Caldwell and Ricotta, 1987; Eleftheriadis *et al.*, 1996a, 1996b; Kotzampassi *et al.*, 2000). In addition, the insufflation gas present in the abdominal cavity (e.g., CO_2) will diffuse through the apical surface of the mesothelial cells to the bloodstream, decreasing the mesothelial pO_2 and inducing hypercarbia and acidosis if not adequately corrected by the assisted ventilation. During standard laparoscopy (100% CO_2 pneumoperitoneum), mesothelial cells do not receive sufficient oxygen supply from the capillaries and the pure CO_2 present in the abdominal cavity diffuses into the mesothelial cells. During laparoscopy with CO_2 pneumoperitoneum with 3% of oxygen, mesothelial cells do not receive sufficient oxygen from the capillaries either, but since the insufflation gas has a pO_2 of 23 mm Hg, which is remarkably similar to the normal intracellular pO_2, they could absorb the oxygen present in the abdominal cavity, raising the intracellular pO_2 up to more physiologic levels. During laparoscopy with CO_2 pneumoperitoneum with 12% of oxygen, mesothelial cells do not receive adequate oxygen supply from the capillaries either, but since the insufflation gas has a pO_2 of 92 mm Hg the oxygen would diffuse into the mesothelial cells increasing the intracellular pO_2 to levels higher than normal.

The hypothesis of peritoneal hypoxia is supported by the absence of CO_2 pneumoperitoneum-enhanced adhesions in mice deficient for some factors regulated by cellular hypoxia, such as hypoxia inducible factors (HIFs) (i.e., HIF-1 and HIF-2) (Molinas et al., 2003b), PAI-1 (Molinas *et al.*, 2003c) and members of the vascular endothelial growth factor (VEGF) family (i.e. VEGF-A, VEGF-B and placental growth factor (PlGF)) (Molinas *et al.*, 2003a), and by blocking pneumoperitoneum-enhanced adhesions in mice treated with antibodies against placental growth factor (Molinas *et al.*, 2003a) and against the VEGF receptor 1 (Flt-1) (Molinas *et al.*, 2004a). Other studies in rats demonstrate that the intraperitoneal administration of small interfering RNA (siRNAs), which downregulate HIF-1 and PAI-1 expression, prevents postoperative adhesions, confirming the role of hypoxia, HIF-1 and PAI-1 (Segura *et al.*, 2007).

The key role of peritoneal hypoxia is also supported by the report of tissue pO_2 measured with a flexible micro-catheter implanted in the abdominal wall in rats in which it was demonstrated that both CO_2 and helium pneumoperitoneum decreases the pO_2 to about 5 mm Hg whereas insufflation with a non-hypoxic gas mixture (80% CO_2 and 20% O_2) induces no significant changes (Wildbrett *et al.*, 2003).

Although the suggested CO_2 pneumoperitoneum-induced peritoneal hypoxia was not confirmed by other authors using a non-injured peritoneum laparoscopic mouse model (Bourdel *et al.*, 2007), subsequent studies of the same group, using the same model with both injured and non-injured peritoneum, clearly demonstrate peritoneal tissue and cellular hypoxia during CO_2 pneumoperitoneum at high insufflation pressures at both the injured and the distant peritoneal sites (Matsuzaki *et al.*, 2010).

Pneumoperitoneum-induced acidosis/hypercarbia as a driving mechanism

The relation between CO_2 pneumoperitoneum-induced acidosis/ hypercarbia and adhesion formation has been addressed in a laparoscopic mouse model in which animals with endotracheal intubation were mechanically ventilated with different patterns (Molinas *et al.*, 2004b). In a first series of experiments, mice were exposed to pure CO_2 pneumoperitoneum during laparoscopic surgery for induction of adhesions, which were evaluated on postoperative day 7. In a second series of experiments, mice were exposed to anaesthesia only or to anaesthesia plus CO_2 pneumoperitoneum and arterial blood gases were measured at the end of the procedures. Adhesions formation was higher in animals poorly ventilated and decreased with higher ventilation rates (ml/min). In comparison with animals who underwent anaesthesia only, the CO_2 pneumoperitoneum increases the pCO_2 and decreases the pH, as has been reported in pigs (Liem *et al.*, 1996), dogs (Kotzampassi *et al.*, 1993), rabbits (Mynbaev *et al.*, 2002), rats (Hazebroek *et al.*, 2002a) and humans (Neuberger *et al.*, 1996). These effects were more pronounced in mice poorly ventilated and counteracted by appropriate ventilation (i.e., higher ventilation rates).

These data demonstrate an association between CO_2 pneumoperitoneum-induced acidosis/hypercarbia and adhesion formation. The mechanism whereby this acidosis/hypercarbia becomes a

cofactor in adhesion formation remains unclear. CO_2 pneumoperitoneum induces respiratory acidosis that, if not corrected, leads to metabolic acidosis and metabolic hypoxia (Mynbaev *et al.*, 2002). This could enhance the ischemic hypoxia in the peritoneum, which was suggested to be a driving mechanism in CO_2 pneumoperitoneum-enhanced adhesion formation. Obviously a direct effect of acidosis/hypercarbia upon cells and molecules involved in adhesion formation cannot be excluded. Indeed, acidosis affects lymphocyte and macrophage functions altering cellular and humoral immune function (Lardner, 2001) and up-regulates VEGF expression independently from hypoxia (Fukumura *et al.*, 2001), which has been reported to be involved in adhesion formation (Molinas *et al.*, 2003a, 2004a; Rout *et al.*, 2000; Saltzman *et al.*, 1996; Wiczyk *et al.*, 1998).

Although these kind of studies are difficult to reproduce in humans and that the clinical significance of the data is unclear, the importance of CO_2 pneumoperitoneum-induced acidosis/hypercarbia should be taken into account for patients in steep Trendelenburg position or with limited cardiovascular adaptation, such as obese and heavy smoker patients, and for laparoscopic surgery of the retroperitoneum or of long duration.

Pneumoperitoneum-induced desiccation and temperature's changes as driving mechanisms

The abdominal insufflation with the standard dry and cold CO_2 for creating the pneumoperitoneum determines that the gas entering the cavity will be warmed to reach an equilibrium in temperature (between the cold gas and the warm peritoneum) and will be humidified to reach and equilibrium in humidity (between the dry gas and the wet peritoneum). Both processes occur at expenses of the patient and more specifically of the peritoneum. Indeed, the peritoneum will lose temperature and water to reach the equilibrium, which consumes energy and consequently induces hypothermia in the patient (Bessell *et al.*, 1995; Bessell *et al.*, 1999). The energy required to warm the cold gas (0.00003 cal to heat 1 mL of CO_2 by 1°C) is much less than the energy needed to humidify the dry gas (577 cal to vaporize 1 g of water) (Binda *et al.*, 2006). Therefore, the pneumoperitoneum-induced hypothermia is to a large extent caused by the pneumoperitoneum-induced desiccation, both effects being intimately associated.

Desiccation of the peritoneum will tends to continue till the pneumoperitoneum reaches 100% of relative humidity (amount of water held by a gas in relation with the maximum amount that can be hold at certain temperature). Hence, it will depend on the time of exposure to the insufflation gas (i.e., more desiccation with longer times), the flow rate through the abdominal cavity (i.e., more desiccation with more gas leakage) and the temperature of the insufflation gas. The effect of this later factor is crucial because the absolute humidity of a gas (mg of water in a litre of gas) is higher at higher temperatures and therefore 100% relative humidity will only be reached with more water (i.e. the higher the insufflation gas temperature the more the water will be needed to reach the equilibrium, and thus the more desiccation will occur).

It was postulated that the desiccation caused by the dry and cold CO_2 pneumoperitoneum will favour the development of peritoneal adhesions. *In vitro* studies confirm that the degree of desiccation depends on the flow rate of the gas through the humidified surface. Indeed, when dry and cold CO_2 circulates through water-filled flaks water is lost depending on the flow rate; the higher the flow the more desiccation is observed (Yesildaglar *et al.*, 1999). Knowing that desiccation is flow-dependent, the effect of dry CO_2 with different flow rates through the abdominal cavity upon adhesion formation was evaluated in rabbits (Yesildaglar and Koninckx, 2000) and mice (Yesildaglar *et al.*, 1999). Since adhesion formation increases with higher flow rates, the key role of desiccation in the pathogenesis of the process was evident. However, because desiccation and hypothermia are intimately linked, the specific contribution of each factor to adhesion formation was difficult to determine.

During a series of experiments performed in a laparoscopic mouse model it become accidentally apparent that mice with lower body temperatures develop fewer adhesions than normothermic mice. Since these totally unexpected observations were very provocative, the effect of body temperature upon adhesion formation was investigated more deeply in subsequent studies.

To address the specific effect of hypothermia without the confounding effect of desiccation, we performed a study using humidified insufflation gas and strictly controlling mouse body temperature throughout the entire surgery. The reduction of adhesion formation with hypothermia was confirmed (Binda *et al.*, 2004). Consistent with these observations, other animal data demonstrated that peritoneal infusion with cold saline decreased postoperative adhesions (Fang *et al.*, 2010), whereas irrigation with warm saline increased postoperative adhesions (Kappas *et al.*, 1988). In humans, local hypothermia after laparotomy was reported to decrease the inflammatory reaction and to increase intestinal peristalsis, thus decreasing adhesion formation (Gataullin *et al.*, 1971).

To address the pure effect of desiccation without the normally associated hypothermia we have designed an experiment in mice in which animals exposed to dry and cold CO_2 pneumoperitoneum with different flow rates through the abdominal cavity (for having different level of desiccation) were protected against hypothermia by covering them with warmed body blankets. As expected, adhesion formation increases with desiccation. Furthermore, this desiccation-induced adhesion formation was prevented by using humidified gas (Binda *et al.*, 2006), which was confirmed by other groups in rats (Peng *et al.*, 2009). Interestingly, this desiccation-induced adhesion formation was also reduced by leaving the animals to develop the normally associated hypothermia (Binda *et al.*, 2006), indicating that both desiccation and hyperthermia contributes independently to adhesion formation. In addition, this experiment also confirmed excellent humidifying capacity of the peritoneal cavity since the intraperitoneal relative humidity of mice in all groups with dry insufflation gas was 100%.

Several mechanisms might be involved in this beneficial effect of hypothermia and detrimental effect of desiccation. Hypothermia might reduce adhesion formation by protecting tissues and cells from the pneumoperitoneum-induced hypoxia, since cell oxygen consumption decreases with temperature. Indeed, hypothermia decreases the global cerebral metabolic rate during ischaemia, slowing the breakdown of glucose, phosphocreatine and ATP and the formation of lactate and inorganic phosphate (Erecinska *et al.*, 2003). In addition, hypothermia reduces the production of ROS during reperfusion in several tissues and organs (Horiguchi *et al.*, 2003; Prasad *et al.*, 1992; Zhao *et al.*, 1996), improves the recovery of energetic parameters during reperfusion (Erecinska *et al.*, 2003), and suppresses the inflammatory response decreasing the infiltration of PMN cells and the production of tumour necrosis factor-a, interleukin-1b and macrophage inflammatory protein-2 (Kato *et al.*, 2002; Patel *et al.*, 2000).

This hypothesis of desiccation as a driving mechanism in adhesion formation is supported by the data demonstrating that the dry and cold CO_2 pneumoperitoneum alters the morphology of the mesothelium (i.e., destroys the hexagonal pattern, reduces the microvilli and bulges up the cells) (Hazebroek *et al.*, 2002b; Mouton *et al.*, 1999; Suematsu *et al.*, 2001; Volz *et al.*, 1999), which can favour the development of postoperative adhesions.

Discussion and conclusions

If these pneumoperitoneum-induced changes contributes to adhesion formation because of purely local effects or because of more general and systemic effects are still difficult to determine. There is emerging evidence suggesting that the pathogenesis of peritoneal adhesion formation is not restricted to the operative site (classical model) and that the entire peritoneal cavity, and maybe the entire organism, could be involved. Consistent with this hypothesis it was recently demonstrated in mice that adhesion formation increases at the lesion site (i.e., uterine horns and pelvic side walls) when the distant peritoneum (i.e., omentum and bowels) is manipulated and that this effect depends on the severity of the manipulation (Schonman *et al.*, 2009).

Although most of these data derives from small animal studies, the relevance of the peritoneal environment in the pathogenesis of adhesion formation is clear and the importance of its proper modulation for adhesion prevention becomes evident.

Pneumoperitoneum-induced peritoneal hypoxia can be reduced by adding 3% of oxygen to the CO_2 pneumoperitoneum, whereas its direct consequences, such as the up-regulation of HIFs, PAI-1, VEGF, can be prevented by specific antibodies against these factors. Some preliminary data in humans indicate that the addition of oxygen could also have some beneficial effects in terms of postoperative pain reduction (unpublished observations).

Pneumoperitoneum-induced ischemia-reperfusion generate ROS, which can be minimize by reducing the insufflation pressure (Kaya *et al.*, 2002; Sare *et al.*, 2002) or by "ischemic preconditioning", a concept that consists of short periods of inflation and deflation upon establishment of pneumoperitoneum (Cevrioglu *et al.*, 2004; Sahin *et al.*, 2007).

Pneumoperitoneum-induced peritoneal desiccation can be reduced by humidifying the insufflation gas, which in addition to reduce adhesion formation will have other local and systemic beneficial effects, such as less destruction of the mesothelium, less postoperative pain and less hypothermia.

The alteration of the peritoneal surface reported in mice, rats and pigs depends on the time of exposure, the insufflation pressure, and the type of insufflation gas (Hazebroek *et al.*, 2002b; Mouton *et al.*, 1999; Suematsu *et al.*, 2001; Volz *et al.*, 1999) and are highly influenced by the temperature and humidity of the insufflation gas. Indeed, some animal studies shows less peritoneal damage with warm and humidified CO_2 (Erikoglu *et al.*, 2005; Mouton *et al.*, 1999; Peng *et al.*, 2009). This protective effect is, however, not conclusive because others studies failed to reach the same conclusions (Glew *et al.*, 2004; Hazebroek *et al.*, 2002b).

The dry and cold gas used for creating the pneumoperitoneum was claimed to contribute to postoperative pain because the dominant source of pain

and discomfort after laparoscopy comes from the peritoneum rather than from the skin or the abdominal wall (Helvacioglu and Weis, 1992). Several human studies have demonstrated that the use warm and humidified insufflation gas is associated with less postoperative pain and analgesic requirements (Sajid *et al.*, 2008; Sammour *et al.*, 2008). In addition, other studies reported that warm and humidified gas reduces the recovering room stay (Ott *et al.*, 1998) and the hospitalisation length (Savel et al., 2005), and that it is associated with a faster return to normal activities (Mouton *et al.*, 1999).

Human data also confirms that the CO_2 pneumoperitoneum-induced hypothermia is prevented with warm and humidified insufflation gas (Sajid *et al.*, 2008). However, there are some evidences that cold and humidified CO_2 is as efficaciously as warm and humidified gas for preventing hypothermia (Schlotterbeck *et al.*, 2008).

Since the side effects of the hypothermia are well known (Insler and Sessler, 2006), surgeons, anaesthesiologists and nurses working in the operating room have always pretended to keep the patient normothermic. Recent data, however, demonstrated that keeping the abdominal cavity slightly cold (i.e., 32°C) and humidified rather than warm and humidified can be useful, or even better, for preventing adhesion formation (Binda *et al.*, 2004; Binda *et al.*, 2006; Fang *et al.*, 2010; Kappas *et al.*, 1988) because this local hypothermia at the trauma site will minimize the local inflammation, and the toxic effects of the hypoxia and of the ischemia-reperfusion processes. The optimal way to achieve this challenging and provocative approach (i.e., normothermic patient with slightly cold abdominal cavity) still has to be determined.

Our studies in the laparoscopic mouse model demonstrate that good surgical technique, avoiding as much as possible collateral damage and unnecessary manipulation, and which can only be achieved by appropriate training, reduces adhesion by some 50%, whereas CO_2 pneumoperitoneum conditioning (i.e., humidification, addition of 3-4% of oxygen, and/or slight cooling) has an additional preventive effect (50% for partial conditioning and another 50% for full conditioning) (Fig. 2) (Binda *et al.*, 2004; Binda *et al.*, 2006; Binda and Koninckx, 2009). The efficacy of adhesion prevention agents were also investigated in mice in two different models, the standard CO_2 pneumoperitoneum and the conditioned CO_2 pneumoperitoneum. The available data already demonstrate that the effects of most products varies with the model (Binda *et al.*, 2007a, 2007b, 2009), indicating that the role of the peritoneal environment is crucial not only for adhesion formation but also for adhesion prevention. Interestingly, it was demonstrated that the combination of good surgical technique, conditioned pneumoperitoneum and some

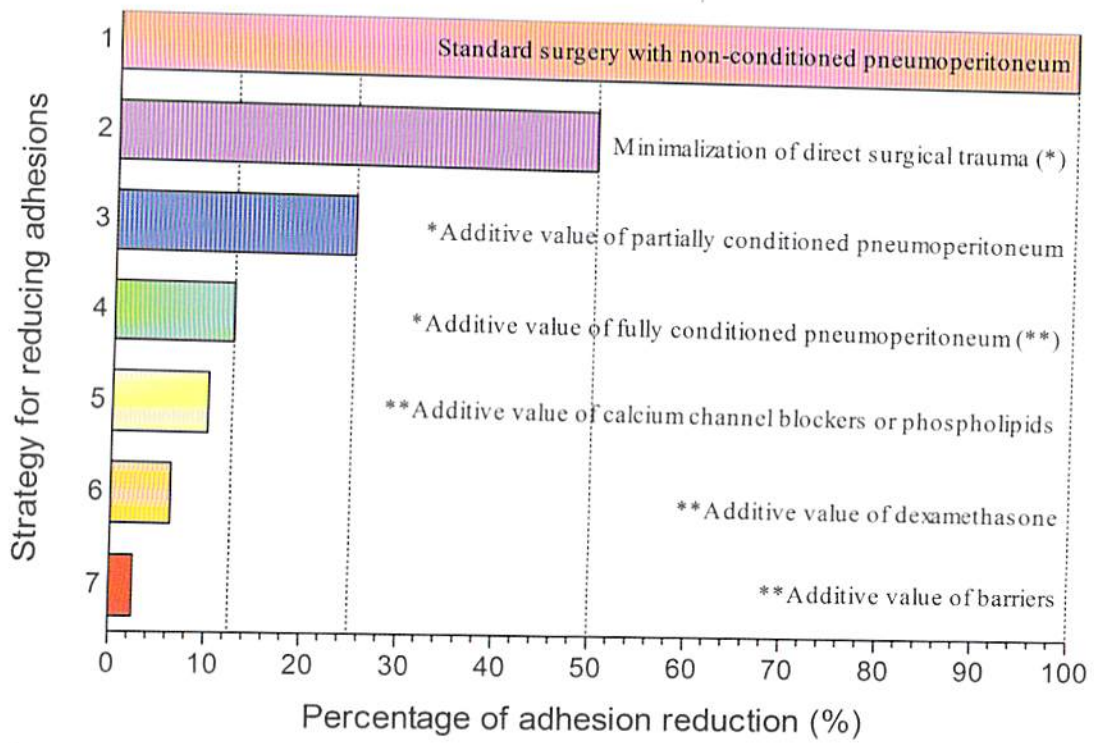

Fig. 2. — Strategies for reducing adhesions (data from a laparoscopic mouse model)
From the maximum adhesions that can be observed in the model (**1**) good surgical technique (reduction of collateral damage and unnecessary manipulation) reduces adhesions by some 50% (**2**). Partial pneumoperitoneum conditioning (addition of 3% of oxygen, humidification or slight cooling) further reduces adhesions by another 50% (**3**), whereas with full pneumoperitoneum conditioning an additional 50% of reduction is observed (**4**). From this already reduced amount of adhesions calcium channel blockers or phospholipids (**5**), dexametasone (**6**) or hyalobarrier gel (**7**) provide some additive effect.

adhesion prevention agents (e.g., calcium channel blocker, phospholipids, dexametasone, Hyalobarrier gel) can reduce adhesion formation up to some 90% (Fig. 2) (Binda and Koninckx, 2009).

In conclusion, there is growing evidence indicating that in addition to the direct surgical trauma the entire peritoneal environment plays a key role in postoperative adhesion formation (Fig. 1), although it alone would not be able to induce *de novo* adhesions. The importance of the peritoneal environment is crucial for both adhesion formation and adhesion prevention after both open and laparoscopic surgery. The important role of the peritoneal cavity environment only recently became apparent and is not yet incorporated in adhesion reducing strategies. The practical implications of this peritoneal environment are more relevant for laparoscopy because the pneumoperitoneum, being a closed environment, can be evaluated, modulated and conditioned more easily than the open environment at laparotomy. Although human studies are lacking, animal data indicate that the standard dry and cold CO_2 pneumoperitoneum is a cofactor in adhesion formation because it induces hypoxia, acidosis and desiccation. The pure effect of desiccation, however, is underestimated because it is associated with hypothermia, which surprisingly has a protective effect against adhesion formation. Our data in the laparoscopic mouse model indicate that peritoneal adhesions can be reduced to a large extent with a proper surgical technique, with adequate pneumoperitoneum conditioning (i.e., addition of 3% of oxygen, humidification and slightly cooling of the insufflation gas) and with the use of adhesion prevention agents (Fig. 2). They also indicate that the efficacy of these agents varies according to the local environment. The ideal "low" temperature for the pneumoperitoneum and the optimal approach to keep the patient normothermic while having a moderate local hypothermia remains to be elucidated. The relevance of all these strategies for peritoneal environment conditioning to reduce adhesion formation and the efficacy of adhesion prevention agents under specific peritoneal conditions still have to be confirmed in larger animals and in humans. If the key role of the peritoneal environment is confirmed in humans, research and strategies for improving patient compliance (e.g., postoperative pain) in general and for adhesion prevention in particular will be directed more to conditioning the peritoneal cavity than to the use of agents.

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