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DEBATE—continued

Reactive oxygen species and adhesion formation

Clinical implications in adhesion prevention

M.M.Binda[1,3], C.R.Molinas[1,2] and P.R.Koninckx[1,2]

[1]Department of Obstetrics and Gynaecology and [2]Centre for Surgical Technologies, University Hospital Gasthuisberg, Katholieke Universiteit Leuven, Leuven, Belgium

[3]To whom correspondence should be addressed at: Department of Obstetrics and Gynaecology, University Hospital Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium. E-mail: MariaMercedes.Binda@uz.kuleuven.ac.be

Postoperative adhesion formation is a major clinical problem. It has been demonstrated that the pneumoperitoneum used during laparoscopy is a cofactor in adhesion formation. Reactive oxygen species (ROS) are produced in a hyperoxic environment and during the ischaemia/reperfusion process. ROS activity is deleterious for cells, which protect themselves by an antioxidant system known as ROS scavengers. ROS activity can increase by up-regulation of ROS themselves or by down-regulation of ROS scavengers. Recent data also point to a role for ROS in adhesion formation since the administration of ROS scavengers decreases adhesion formation in several animal models. ROS activity increases during both laparotomy and laparoscopy. During laparoscopy, the pneumoperitoneum determines ischaemia at the time of insuflation and reperfusion at the time of deflation. During laparotomy, the environment has a 150 mmHg partial pressure of oxygen (pO_2), which is much higher than the intracellular pO_2 (5–40 mmHg). This can explain the increase in ROS activity. The aim of this debate is to open a discussion about the importance of ROS activity, besides the known players and mechanisms involved, in adhesion formation and in adhesion prevention.

Key words: adhesion formation/antioxidants/free radical scavengers/pneumoperitoneum/reactive oxygen species

Introduction

Following surgery, adhesions form in >80% of women and can cause female infertility (Drake and Grunert, 1980), intestinal obstruction (Ellis, 1997), chronic pelvic pain (Duffy and diZerega, 1996) and difficulties at the time of reoperation. The burden of postoperative adhesions is best illustrated by the study showing that 35% of women having open gynaecological surgery will be readmitted on average 1.9 times in the following 10 years for reoperation due to adhesions (Ellis, 2000).

Pathophysiology of intraperitoneal adhesions

Peritoneal injury, due to surgery, infection or irritation, initiates an inflammatory reaction that increases peritoneal fluid, including proteins and cells. This fibrinous exudate leads to formation of fibrin (Holmdahl, 2000), by activation of the coagulation cascade, which transforms prothrombin (Factor II) into thrombin (Factor IIa). Thrombin then triggers the conversion of fibrinogen into monomers of fibrin, which interact and polymerize. The initially soluble polymer becomes insoluble by coagulation factors such as Factor XIIIa and is deposited on the wound surface (diZerega, 2000). Within this fibrinous exudate, polymorphonuclears (PMN), macrophages, fibroblasts and mesothelial cells migrate, proliferate and/or differentiate. Macrophages increase in number and change functions, e.g. more accurate phagocytosis, greater respiratory burst activity and secretion of a variety of substances that recruit mesothelial cells onto the injured surface. Mesothelial cells form islands throughout the injured area, proliferate and cover the denuded area. All these cells release a variety of substances such as plasminogen system components, arachidonic acid metabolites, reactive oxygen species (ROS), cytokines and growth factors such as interleukins (IL), tumour necrosis factor α (TNFα), transforming growth factors α and β (TGFα and TGFβ). These factors modulate the process of peritoneal healing and adhesion formation at different stages.

The fibrinous exudate and fibrin deposition is an essential part of normal tissue repair, but its complete resolution is required for normal healing. The degradation of fibrin is regulated by the plasminogen system. The inactive proenzyme plasminogen is converted into plasmin by tissue-type plasminogen activator (tPA) and/or urokinase-type plasminogen activator (uPA), which are inhibited by the plasminogen activator inhibitors 1 (PAI-1) and 2 (PAI-2). Plasmin is a serine protease which degrades fibrin into fibrin degradation products. Plasmin has, in addition, a role in other stages of tissue repair, e.g. extracellular matrix (ECM) degradation, activation of

proenzymes of the matrix metalloprotease (MMP) family, and activation of growth factors. Plasmin can be directly inhibited by plasmin inhibitors, i.e. α_2-macroglobulin, α_2-antiplasmin and α_1-antitrypsin, but their role in peritoneal fibrinolysis is not well defined (Holmdahl, 2000).

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. Fibroblasts will invade the fibrin matrix and ECM will be produced and deposited. This ECM is normally completely degraded by MMPs, leading to normal healing. If this process is inhibited by tissue inhibitors of MMPs (TIMPs), peritoneal adhesions will be formed. In addition to fibroblast invasion and ECM deposition, the formation of new blood vessels has been universally claimed to be important in adhesion formation.

During peritoneal healing, cell–cell interactions between mesothelial cells, macrophages and also fibroblasts contribute to the healing of the peritoneum. Adhesion fibroblasts have developed a specific phenotype. Compared with normal peritoneal fibroblasts, adhesion fibroblasts have increased basal levels of collagen I, fibronectin, MMP-1, tissue MMP-1, TGFβ, PA-1, IL-10 and decreased levels of tPA (Saed *et al*., 2001).

Pneumoperitoneum-enhanced adhesion formation

Laparoscopy, in comparison with laparotomy, was claimed to be less adhesiogenic, but the data are not conclusive (Pouly and Seak-San, 2000). In recent years the effects of CO_2 pneumoperitoneum have become increasingly scrutinized. CO_2 pneumoperitoneum induces adverse effects such as hypercarbia, acidosis (West *et al*., 1997), hypothermia and desiccation (Gray *et al*., 1999). It alters peritoneal fluid (Ott, 2001) and the morphology of the mesothelial cells (Volz *et al*., 1999; Hazebroek *et al*., 2002). Pneumoperitoneum is a cofactor in adhesion formation since adhesions increase with the duration of the pneumoperitoneum and with the insufflation pressure in rabbits (Ordonez *et al*., 1997; Yesildaglar and Koninckx, 2000) and mice (Yesildaglar *et al*., 1999; Molinas *et al*., 2001). This pneumoperitoneum-enhanced adhesion formation has been suggested to be mediated by mesothelial hypoxia because similar effects were observed with helium pneumoperitoneum because the addition of 2–4% of oxygen to both CO_2 and helium pneumoperitoneum decreased adhesion formation (Molinas and Koninckx, 2000; Molinas *et al*., 2001) and because this effect was absent in mice deficient for hypoxia inducible factor (HIF) (Molinas *et al*., 2003a), for PAI-1 (Molinas *et al*., 2003b), for vascular endothelial growth factor (VEGF) or for placental growth factor (PlGF) (Molinas *et al*., 2003c).

Reactive oxygen species

Reactive oxygen species are produced in a series of conditions such as cells maintained under hyperoxic conditions (Bostek, 1989) and during reperfusion following ischaemia (Eleftheriadis *et al*., 1996; Glantzounis *et al*., 2001). They are also produced during a bactericidal immune response (Babior *et al*., 1973). ROS comprises free radicals and non-free radicals. Free radicals, i.e. superoxide anion (O_2^-), hydroxyl radical (·OH) and nitric oxide (NO), are unstable atoms or molecules with an unpaired electron: they take an electron from other stable molecules to stabilize themselves, thus causing a chain reaction by destabilizing other molecules. Non-free radicals, i.e. hydrogen peroxide (H_2O_2), have paired electrons but by their natural instability they can easily become free radicals. Since ROS are deleterious for cells, they protect themselves by an antioxidant system known as ROS scavengers. These comprise antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) (Forman and Torres, 2002) and antioxidant molecules such as glutathione, carotenoids, retinoic acid, ascorbic acid and vitamin E. The balance between ROS and ROS scavengers will determine ROS activity and toxicity.

ROS inhibit cellular proliferation and produce cellular senescence (Honda *et al*., 2001, 2002), induce molecular damage of molecules such as DNA, proteins and lipids (Wei and Lee, 2002), and cause ageing (Wei and Lee, 2002) and apoptosis (Taglialatela *et al*., 1998). ROS are also involved in a variety of diseases such as in the inflammatory reaction associated with endometriosis (Ota *et al*., 1999; Van Langendonckt *et al*., 2002), in neurodegenerative diseases as Alzheimer's (Nourooz-Zadeh *et al*., 1999), in autoimmune diseases as systemic lupus erythematosus (Ahsan *et al*., 2003) and in the pathogenesis of diabetic nephropathy (Ha and Lee, 2001). ROS have also been associated with surgery and postoperative adhesion formation (Tsimoyiannis *et al*., 1989; Portz *et al*., 1991; Hemadeh *et al*., 1993; Galili *et al*., 1998; Taskin *et al*., 1999).

Surgery and reactive oxygen species

During open surgery, an increase in ROS production has been reported, i.e. an increase of superoxide anions (Elkins *et al*., 1991), of xanthine oxidase, an enzyme involved in ROS formation (Anup *et al*., 1999), and of malondialdehyde, a marker of ROS production (Souza *et al*., 2003). Also during laparoscopic surgery, ROS increase. Indeed, recent findings showed an increase of markers of ROS production such as 8-isoprostaglandin $F_{2\alpha}$ and hydroxyeicosatetranoic acid in human peritoneum in a time- and CO_2 volume-dependent manner (Souza *et al*., 2003) and of malondialdehyde in the intestine, liver and lung in rats (Eleftheriadis *et al*., 1996). In addition, a negative correlation between ROS scavengers such as GSH-Px, SOD, CAT and GSH, and duration/amount of CO_2 exposure was observed (Taskin *et al*., 1998, 1999). Since abdominal insufflation/deflation causes ischaemia/reperfusion (Caldwell and Ricotta, 1987; Kotzampassi *et al*., 1993; Shuto *et al*., 1995; Eleftheriadis *et al*., 1996; Gutt and Schmandra, 1999; Kotzampassi *et al*., 2000; Schmandra *et al*., 2001), which is well known to generate ROS (Glantzounis *et al*., 2001), a causal role of the pneumoperitoneum, especially at high

insufflation pressure in increased ROS production, can be postulated.

Adhesion formation and reactive oxygen species

It has been suggested that ROS can be involved in postoperative adhesion formation but direct data to support this are scant. It has been demonstrated *in vitro* that free radicals contribute to the formation of cross-linked proteins that may serve as an initial scaffolding for the development of adhesions frequently seen in joints (Dijkgraaf *et al*., 2003). In humans, in comparison with microlaparoscopy (2 mm endoscope, local anaesthesia and 10 mmHg insufflation pressure), standard laparoscopy (10 mm endoscope, general anaesthesia and 15 mmHg insufflation pressure) was reported to be associated with a higher amount of CO_2 used, with decreased levels of ROS scavengers, i.e. GSH-Px, SOD, CAT and GSH, and with increased adhesion formation (Taskin *et al*., 1999).

Indirectly, a role for ROS in adhesion formation is derived from the observation that ROS scavengers reduce adhesion formation following open surgery in different animal models. Indeed, CAT, SOD and trimetazidine reduce adhesion formation induced by vascular obstruction/reperfusion of an ileal segment in rats (Tsimoyiannis *et al*., 1989, 1990); CAT and SOD also reduce adhesion formation in an endometriosis model in rabbits (Portz *et al*., 1991). In addition, intraperitoneal administration of methylene blue reduces adhesion formation induced by scraping the uterus in rats (Galili *et al*., 1998); intraperitoneal administration of melatonin also prevents adhesion formation induced by monopolar cautery in rats (Özçelik *et al*., 2003). Similarly, oral supplements of vitamin E reduce adhesion formation created by scraping the caecum with mesh gauze in rats (Hemadeh *et al*., 1993). This effect of vitamin E, however, was not confirmed by denuding the serous surface of the uterus in rats (Sanfilippo *et al*., 1995).

Finally, the observation that adhesion formation decreases by adding 2–4% of oxygen to the CO_2 pneumoperitoneum (Molinas and Koninckx, 2000; Molinas *et al*., 2001) could be explained by the fact that this addition of oxygen prevents the decrease of ROS scavengers, and thus the increase of ROS activity.

Discussion

Research in adhesion formation and prevention has been performed with the dogma that laparotomy is the standard and with the assumption that the mechanisms involved in adhesion formation following laparotomy and laparoscopy are comparable. Recent data unequivocally demonstrated a role of the pneumoperitoneum through mechanisms involving HIF, the plasminogen system, i.e. PAI-1, and the VEGF family, i.e. VEGF-A, VEGF-B and PlGF. Simultaneously, accumulating data point to a role for ROS in adhesion formation. Interestingly, an increase in ROS activity has been shown following both laparotomy (Elkins *et al*., 1991; Anup *et al*., 1999; Souza *et al*., 2003) and laparoscopy (Eleftheriadis *et al*., 1996; Taskin *et al*., 1998, 1999; Glantzounis *et al*., 2001; Souza *et al*., 2003). The mechanisms underlying the increased ROS activity could, however, be different. During laparoscopy, ROS scavengers can decrease by hypoxia, whereas after laparoscopy ROS can increase by the ischaemia–reperfusion process. During laparotomy, ROS activity increases, and we suggest that this is due to a hyperoxic environment of the peritoneum. Indeed, during laparotomy the peritoneum is exposed to air with a pO_2 of 150 mmHg whereas the normal pO_2 for peripheral cells is estimated between 5 and 40 mmHg (Guyton and Hall, 2000).

The importance of these observations on ROS activity is that the traditional concepts of adhesion formation, involving tissue trauma, fibrin deposition and fibrinolysis, fibroblast invasion, ECM deposition and angiogenesis, have to incorporate the effect of the environment upon the peritoneal cells, e.g. mesothelial cells, macrophages, fibroblasts in order to understand the differences between laparotomy and laparoscopy. During laparotomy, the pO_2 of the environment is clearly hyperoxic for the peritoneal cells. During laparoscopy, the CO_2 pneumoperitoneum creates a hypoxic environment. Specifically relevant for adhesion formation is that a hypoxic environment induces irreversible molecular changes in peritoneal fibroblast, such as increases in cyclooxygenase 2 (COX-2) (Saed *et al*., 2003), ECM (Saed and Diamond, 2002), and PAI-1 (Saed and Diamond, 2003); moreover, hypoxia modulates the expression of TGFβ1, -2 and -3 and their receptors (Saed *et al*., 2002) and decreases tPA (Saed and Diamond, 2003).

These concepts are fundamental for the clinically important problem of adhesion prevention. Until now, prevention has focused on good surgical techniques minimizing tissue trauma and fibrin deposition and on mechanical separation of surfaces and upon fibrinolysis. The understanding of the role of the pO_2 in adhesion formation and of the mechanisms involving ROS, hypoxia during CO_2 pneumoperitoneum and hyperoxia during laparotomy, and angiogenesis could open new possibilities in adhesion prevention. Recent new approaches in animals, such as the addition of 3% of oxygen to the pneumoperitoneum (Molinas and Koninckx, 2000; Molinas *et al*., 2001), the neutralization of PlGF by monoclonal antibodies (Molinas *et al*., 2003c) and the administration of ROS scavengers (Tsimoyiannis *et al*., 1989, Portz *et al*., 1991; Hemadeh *et al*., 1993; Galili *et al*., 1998; Özçelik *et al*., 2003), open unexpected possibilities for postoperative adhesion prevention.

We fully realize that these concepts are provocative. Yet to stimulate thinking and discussion—'*du choc des idées jaillit la lumière*'—we decided to write this introduction to a Debate.

Indeed, the relative importance of fundamental mechanisms as the fibrinolytic system, angiogenic factors and ROS in adhesion formation is still unclear. Moreover, the role of a fundamental process such as ROS activity is important for many other aspects in medicine e.g. the ischaemia–reperfusion process and transplant surgery, embryo implantation, cell culture, and possibly IVF and embryo culture.

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