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ENDOSCOPIC SURGERY

AND ADHESION FORMATION

IN THE RABBIT MODEL

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Dedicated to

my wife Hilde,

who decided to postpone her own professional projects

in order to accompany me during these years far away from our country,

my daughter Andrea,

whose happiness replaced the missed latino-american sunny days,

and to

my son Alejandro,

who born in Gasthuisberg is going to remind me of

this country, this beautiful city and this prestigious university for the rest of my life.

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PUBLICATIONS AND PRESENTATIONS RELATED WITH THIS THESIS

PUBLICATIONS

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Molinas CR and Koninckx PR. *Hypoxemia induced by CO_2 or helium pneumoperitoneum is a co-factor in adhesion formation in rabbits*. Hum Reprod, submitted.

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CO_2 and helium pneumoperitoneum and the related anoxemia as a co-factor in adhesion formation. A prospective randomised trial in a rabbit model. Combined Meeting of the International Congress on Peritoneal Repair and Adhesions (PAX, Fifth Meeting) and International Mesothelioma Interest Group (IMIG, Fifth Meeting). October 5-8, 1999. Stoke Rochford -United Kingdom. Oral presentation.

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CHAPTER 1

GENERAL INTRODUCTION

Peritoneal injury is the first step of the cascade that leads to postoperative adhesion formation (1-3). Several factors are implicated in this injury and, obviously, the surgical procedures, either by laparotomy or by laparoscopy, are one of them (4). It is universally accepted that surgery performed by less experienced surgeons is longer and more traumatic. More experienced surgeons have more gentle tissue handling and surgery is more precise resulting in less traumatic and more haemostatic surgery with less postoperative adhesions. This emphasises the importance to standardise surgical techniques in order to study adhesion formation. An absolute necessity to achieve this is adequate training. Therefore we first emphasised on training and standardisation of the surgical procedure.

1.1 Training in endoscopy

Since laparoscopy is rapidly replacing laparotomy in both general and gynaecological surgery and since laparoscopic surgery requires specific skills in hand-eye coordination due to the lack of manual contact with the tissue and the restricted instrument mobility, the importance of training is evident.

In vitro and *in vivo* training improves the laparoscopic skills allowing better quality of surgery and less operation time. Training in endotrainers increases the performance of laparoscopic skills as was shown for intra-corporeal and/or extra-corporeal knot tying, suturing, cutting, clipping, and transferring objects with both hands (5-10). Also in animal models, such as the rabbit, the operation time decreased and the quality of surgery increased with training (4). In humans it has been shown that experience gained with training decrease the operative complication rates (11) and the operation time, as was shown for laparoscopic Pomeroy tubal ligation (12), ovarian masses (13) and ectopic pregnancies (14). Experienced surgeons achieve shorter operation times and better quality than inexperienced surgeons and the differences decrease with training (10,12-14).

Since the relation between training, duration and quality of surgery and adhesion formation is clear and since there is not available an easily reproducibly *in vivo* training model, the aim of the first part of this work (Chapters 3 and 4) was the development of a model that permit the improvement of the laparoscopic skills in general and the standardisation of these skills in adhesion formation experiments.

Therefore, the development of the rabbit training model, including a prospective, randomised study to evaluate the effect of training upon duration and quality of surgery, was performed in Chapter 3 and a prospective, randomised study to evaluate the effect of the diameter of the endoscope upon the same variables was performed in Chapter 4.

1.2 Adhesion formation

1.2.1 Incidence and cause of adhesions

Intra-peritoneal adhesions may be classified as congenital or acquired which can be postinflammatory or postoperative. Some 10% of patients without previous surgery have adhesions, 9% postinflammatory and 1% congenital. In contrast, adhesions are found in 94% of patients with at least one previous surgery, 92% postoperative, 1% postinflammatory and

1% congenital (15). These data clearly show the importance of postoperative adhesions; therefore this work will refer mainly to this kind of adhesions.

1.2.2 Clinical significance

Intra-peritoneal adhesions are clinically important because of the associated complaints and complications. They are a major cause of intestinal obstruction in men and women and of chronic pelvic pain and infertility in women.

In developed countries adhesions are the cause of intestinal obstruction in 40% of the cases but this proportion increases to 60-70% if we consider only the small bowel obstructions. The situation is different in the third world where hernias still are the main cause of intestinal obstruction (15). The surgeries that most frequently cause adhesive obstruction are non-elective appendicectomies and gynaecological surgeries (16,17). It is important, however, to remember that these are the most common surgeries performed and, therefore, it remains unclear whether these are more adhesiogenic or whether they only are more frequently performed. The sites of postoperative adhesions differ from the sites of obstructive adhesions. Whereas the omentum and the site of the previous surgery are more commonly involved in adhesions, the adhesions involving the small bowel are more frequently obstructive (16). Intestinal obstruction due to postoperative adhesions may occur at any time. Some studies demonstrated that 1% of patients who underwent surgery developed intestinal obstruction within 1 year of surgery (16) and others reported that the interval was greater than 1 year in 84% of the cases (17).

The relation between adhesions and chronic pelvic pain is controversial, mainly because their association does not imply necessarily a causal relationship. Some studies found adhesions in 26-40% of women with pelvic pain (18-20). A large number of patients with adhesions, however, do not have pelvic pain, as was clearly demonstrated in a retrospective study which reported that 39% of infertility patients who underwent laparotomy had adhesions whereas only 12% of them had pelvic pain (20). This study did not find differences in the location and density of adhesions of the patients with or without pelvic pain whereas another study reported that only the adhesions that restricted organs motion and expandability were associated with pelvic pain (21). At the same time several studies reported a relief of pain in some 78% of patients after adhesiolysis (19).

Intra-peritoneal adhesions are well recognised as a cause of female infertility. Peri-adnexal adhesions were found in some 30% of infertile women (22,23) and pregnancy rates increase significantly after adhesiolysis (24-26). The mechanism probably is the effect of adhesions upon the sweeping of the fimbria over the ovary. Another mechanism, the inhibition of the folliculogenesis, as a consequence of the pressure around the ovaries limiting the follicular growth, is controversial (27).

1.2.3 Pathogenesis

To understand the processes involved in adhesion formation a brief review of the anatomy and physiology of the peritoneum will be given.

The peritoneum, with a surface area of some 10.000 cm^2 in adults, is the largest organ in humans comparable to the skin. It diminishes the friction among abdominal viscera and with the abdominal wall, protects against infections and serves as a reservoir of fat, especially in the omentum. It is composed of 2 layers: a continuous layer of mesothelial cells resting upon a basal lamina and a basement membrane attached to the abdominal wall by areolar tissue (1,28).

The mesothelial cells are highly differentiated, as the pleura and pericardium, and the apical surface contains abundant long microvilli that increase the functional surface of the peritoneum for absorption and secretion. Since these mesothelial cells are poorly interconnected with very loose desmosomes or intercellular bridgings, the membrane is extremely delicate and breakable. Connective tissue proteins and vascular channels provide scaffolding for the mesothelial cells. Interspersed among these connective tissue proteins are poorly differentiated epithelioid-like cells similar to fibroblasts that can undergo a variety of differentiation changes after exposure to a stimulus as peritoneal injury (1,28,29).

The surface of the peritoneum is the key site in adhesion formation because of 2 unique properties: its delicacy and its uniform and relatively rapid re-epithelialization. Its delicacy is due to the lack of interconnections between the mesothelial cells, as described above, and, therefore, any minimal injury of the peritoneum could denude its surface leading to adhesion formation. The re-epithelialization of the surface of the peritoneum is irrespective of the size of injury and is complete in 5-7 days. This process is different for the skin because the entire surface becomes epithelialized from islands of mesothelial cells throughout the injured area and not gradually from the borders (1,30).

The intact peritoneal cavity contains 3-50 ml of fluid with plasma proteins including a large amount of fibrinogen and with a variety of free-floating cells. The cells are predominantly macrophages, lymphocytes, mast cells and desquamated mesothelial cells. The macrophages are so-called resident macrophages and appear to have no biological function. (1,28).

After surgery an inflammatory exudate develops and the amount of peritoneal fluid, cells and plasma proteins increase considerably and the population of white blood cells changes. During the first 2 postoperative days a large number of polymorphonuclears (PMN) enter and, in the absent of infection, depart within the 3-4 days. Macrophages increase significantly in number and change their functions. After day 5, macrophages become the most important component of the leukocyte population. They phagocytize more accurately, have greater respiratory burst activity and secrete a variety of substances including cytokines and growth factors recruiting new mesothelial cells onto the surface of the injury. These cells migrate from the adjacent peritoneum and form small islands throughout the injured area that proliferate into sheets of mesothelial cells. Some authors suggest that the new mesothelial cells are derived from the fibroblastic-like cells within the connective tissue protein matrix that supports the peritoneal surface (1,28,30).

Peritoneal injury produces a complex biological response including the expression and release of cytokines, arachidonic acid metabolites, oxygen-derived free radicals and growth factors, which leads either to the normal peritoneum repair or to adhesion formation. Several systems, such as coagulation, fibrinolysis, kinin/bradykinin, arachidonic acid metabolism and complement, are involved in this process (2).

First, an inflammatory reaction increases exudation that contains fibrinogen. Fibrinogen is transformed into monomers of fibrin by thrombin. The fibrin polymerises, is initially soluble and becomes subsequently insoluble by some coagulation factors, such as Factor XIIIa. The insoluble fibrin polymer interacts with large proteins, such as fibronectin, to produce the fibrin matrix (1).

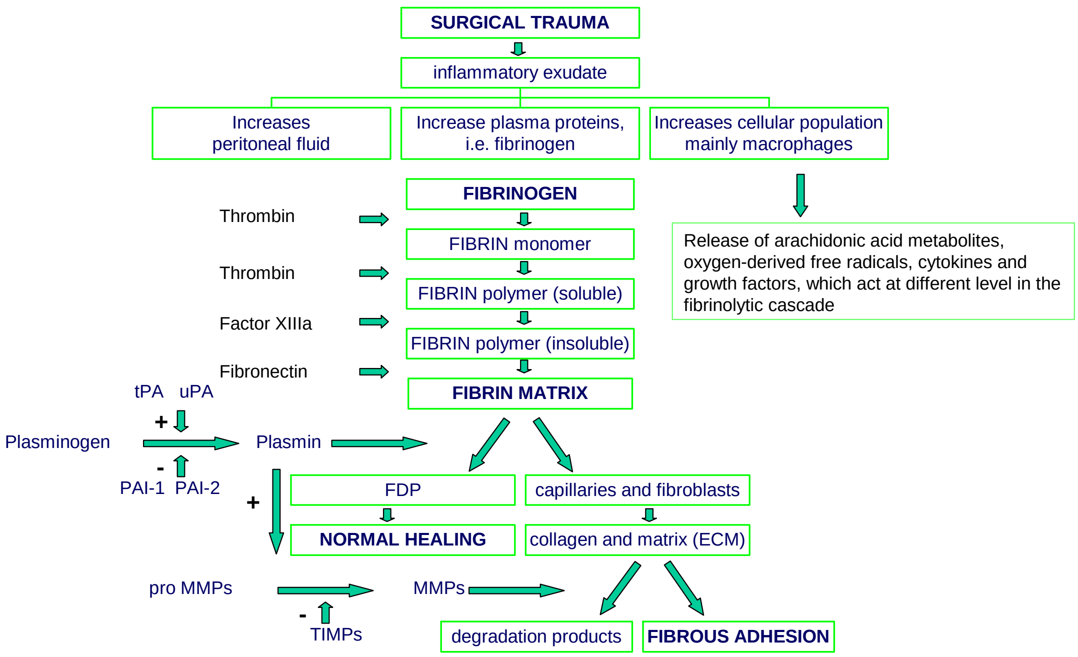
The most important natural defence against adhesion formation is the fibrinolytic system. The function of this system is to lyse the fibrin into fibrin degradation products (FDP) though the action of plasmin. Plasmin is stored in an inactive form, plasminogen, which is converted to its active form by tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). tPA is the main plasminogen activator in blood and uPA in urine. The action of both types of plasminogen activators is counteracted by plasminogen activating inhibitors type 1 (PAI-1) and type 2 (PAI-2). Others plasma proteins, α_2-antiplasmin, α_2-macroglobulin and α_1-antitrypsin, also inhibit plasmin activity. Reduced plasminogen activator activity (PAA) obviously leads to adhesion formation and could be caused by decreased levels of tPA and/or uPA, increased levels of PAI-1 and/or PAI-2, or both (2).

The balance of fibrin deposition and degradation determines adhesion formation. If local fibrinolysis is sufficient, fibrinous adhesions are lysed resulting in complete tissue restitution. On the contrary, if local fibrinolysis is insufficient fibrin remains and acts as a scaffold for capillaries and fibroblasts leading to connective tissue formation with collagen and matrix constituents and finally to permanent fibrous adhesion formation (2).

The extracellular matrix may be remodelled by matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs). Plasmin has also a role in this step since it activates MMPs from their inactive forms or proMMPs. MMPs act upon the extracellular matrix and produce degradation products whereas the TIMPs counteract this action (2,3).

Cytokines and growth factors and their receptors play a key role in normal peritoneal healing and adhesion formation. The growth factors family involved in this process comprise: epidermal growth factor (EGF), heparin-binding EGF, insulin-like growth factors, transforming growth factors α and β (TGF-α and TGF-β), platelet-derived growth factors and fibroblast growth factor. The cytokine family comprise: interleukins (IL), tumor necrosis factor α (TNF-α), granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, macrophage colony-stimulating factor, etc. These cytokines and growth factors, which act at different levels of the fibrinolytic cascade, are synthesized and released by activated macrophages and other cells. Their availability must be optimal, precise and synchronized for a normal wound healing. Inhibition or excess expression of these substances will lead to a failure in normal healing, either impairment (non healing) or excess tissue formation (adhesions) (3).

Fig. 1: Pathogenesis of adhesion formation



1.2.4 Laparoscopy and adhesion formation

In humans, laparoscopy was claimed to be less adhesiogenic than laparotomy. A randomised trial of patients with ectopic pregnancy who underwent surgical treatment by laparoscopy or by laparotomy demonstrated significant fewer postoperative adhesions in the laparoscopy group corroborated during a second look laparoscopy (31). More and larger clinical studies have not been performed because of the obvious difficulties and ethical concerns for follow up and scoring of adhesions. In animals, some studies failed to show differences between laparoscopy and laparotomy in rats (32) and in rabbits (33-34), whereas others did show significantly fewer adhesions after laparoscopy in rats (35), dogs (36), pigs (37,38) and rabbits (39).

Although the surgical trauma is theoretically less during a laparoscopy than during a laparotomy, it is not enough to prevent adhesion formation. On the other hand, it is important to take into account that laparoscopies are performed in a different environment, which could be involved in the development of adhesions. Indeed, CO_2 pneumoperitoneum was recently shown to play a role in peritoneal adhesion formation in rabbits (4) and mice (40). This could be important since CO_2 is generally used for pneumoperitoneum for safety reasons because of its high solubility in water and exchange rate in the lungs. CO_2 induces local changes such as intra-peritoneal acidosis (41-43) and desiccation of mesothelial layers (44), whereas the intra-peritoneal pressure induces adverse effects upon peritoneal microcirculation (45,46) possibly inducing hypoxemia.

Hypoxemia induced by compression of the capillary flow in the superficial peritoneal layers during prolonged pneumoperitoneum could be a key factor in adhesion formation. It is logic to assume that increasing duration of pneumoperitoneum and higher intra-abdominal pressures limit the peritoneal capillary flow increasing hypoxemia and that the addition of oxygen could decrease hypoxemia. This assumption is consistent with the observations that adhesion formation increases with duration of CO_2 pneumoperitoneum (4,40) and with higher intra-abdominal pressures (47). Furthermore, adhesion formation decreases with the addition of oxygen (48).

The effect of other gasses used for pneumoperitoneum upon adhesion formation has been not assessed up to date. Therefore, in order to confirm the hypothesis of the adhesiogenic role of hypoxemia it is necessary to evaluate another gas, such as helium, that does not produce acidosis.

1.2.5 Adhesion prevention

Less surgical trauma during the surgical procedures will obviously diminish adhesion formation but this alone will not prevent adhesions. Several studies reported a variety of approaches to prevent adhesions by reducing the local inflammation, preventing the fibrin deposition, removing the fibrin deposits and separating traumatized surfaces but, up to date, no single approach was proven to be universally effective (28,49).

Reduction of local inflammation could be achieved with systemic and intraperitoneal (IP) corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs). They may reduce the release of fibrinous exudate in response to inflammation at the site of surgery. However, they have 2 main obstacles. First, they have to act specifically against adhesions but not against normal wound healing. Unfortunately many process in adhesion formation, such as exudation, coagulation and fibrinolysis, are involved also in normal re-epithelialization. Second, they need to arrive to the site of surgery and to stay there long enough to act properly. It is well known that the ischemic sites are more vulnerable to adhesion formation but these sites are cut off from the bloodstream and thus from systemic drug delivery. On the other hand, the peritoneum has a very rapid absorption rate for small molecules limiting the half-life and the efficacy of the drugs administered IP. In a large number of experimental and in a few clinical studies, corticosteroids, such as dexamethasone, hydrocortisone, prednisolone and betamethasone, and NSAIDs, such as ibuprofen, tolmetin and oxyphenbutazone, have shown contradictories results in the reduction of postoperative adhesions (28,49,50).

Prevention of fibrin deposition is achieved by several approaches including peritoneal irrigation and adjustments of surgical techniques. Peritoneal irrigation with saline, Ringer's lactate or solutions containing heparin or antibiotics, minimizes tissue desiccation and dilutes or washes away fibrinous exudates, but whereas some authors reported a reduction in adhesion formation in animals and humans others did not found a significant effect. Adjustments of surgical techniques in order to minimize the surgical trauma can be achieved avoiding unnecessary sutures and introduction of foreign material into the body. A variety of animals and human studies have shown that the suture of a peritoneal defect, as well as the use of powder gloves and non-absorbable sutures increase adhesion formation (2,28,49,50).

The removal of the fibrin deposits is achieved with proteolytic enzymes, such as hyaluronidase, pepsin, trypsin, streptokinase and urokinase. However, since fibrin deposition occurs not only in adhesion formation but also in wound healing and haemostasis, the use of these fibrinolytic drugs carries a risk of impaired wound healing and bleeding. Recombinant tissue plasminogen activator (rt-PA) was reported as an effective treatment in animals without deleterious side effects (28,49,50).

Barriers methods have been developed to limit tissue apposition during the 5-7 days required for peritoneal re-epithelialization permitting, theoretically, a normal healing. They can be a mechanical device or a macromolecular solution. An ideal barrier must be biodegradable, safe, non-inflammatory, non-immunogenic and rapidly and easily applied. Mechanical barriers, such as expanded polytetrafluoroethylene (ePTFE) (Preclude™), hyaluronic acid-carboxymethylcellulose (HA-CMC) (Seprafilm™) and oxidized regenerated cellulose (ORC) (Interceed®) and barriers solutions, such as carboxymethylcellulose (CMC), chondroitin sulphate, dextran 70 and hyaluronic acid-phosphate-buffered saline (HA-PBS) (Sepracoat™), have been evaluated with promissory results in several clinical and experimental studies (28,49,50).

Others approaches, such as calcium channel blockers, cytostatics and motility promoters have been used with results not sufficiently clear. The anti-adhesion effect of some antibodies against particular cytokines and growth factors involved in adhesion formation were described recently.

The hormonal therapy for adhesion prevention has been reported in some studies but the overall results remain unclear. Hypoestrogenic status after oophorectomy or after treatment with gonadotrofin releasing hormone analogue (GnRH-a) decreased adhesion formation (51-53). Estrogens might exert this action since it regulates important process in the female genital tract that could have similarities with process involved in adhesion formation, such as angiogenesis in the uterus (54), uterine blood flow (55), uterine growth factors expression (56) and, possibly, proliferation of fibroblasts and mesothelial cells (57). On the contrary, other authors found that estrogens decreased murine monocyte chemoattractant protein-1 (MCP-1), affecting the macrophage recruitment and thus decreasing adhesion formation (58,59). Although the general agreement concerning the anti-inflammatory and immunosuppressive effects of progesterone (60-72), some studies found a reduction in postoperative adhesions after the treatment with natural progesterone (73) or with medroxyprogesterone acetate (MPA) (74,75), a more potent synthetic progestagen, whereas others reported not effects or increased scores of adhesions after either progesterone (76-79) or MPA (72,79) treatment. The antiprogestin mifepristone (RU486) has been associated, as well, with decreased postoperative adhesion formation (52). All these contradictory data indicate that sexual hormones could have an important role in the development of adhesions but the mechanisms are still unclear and demand further studies.

Since the importance of postoperative adhesion formation is clear and since mostly adhesion formation and prevention is studied either *in vitro* or during laparotomies, the aim of the second part of this work (Chapters 5, 6 and 7) was the study of some aspects of postlaparoscopic adhesion formation.

Therefore, prospective, randomised studies were performed to confirm the effect of duration of the CO_2 pneumoperitoneum (Chapter 5), to evaluate the effect of helium pneumoperitoneum and the addition of oxygen (Chapter 6) and to evaluate the effect of an antiprogestin (Chapter 7) upon postlaparoscopic adhesion formation.

CHAPTER 2

GENERAL MATERIAL AND METHODS

2.1 Animals

All experiments were performed in adult, female, New Zealand, white rabbits weighting between 2.7 and 3 kg. The animals were housed at the Centre for Laboratory Animal Care of the Catholic University of Leuven. They were kept under standard laboratory conditions with a temperature of 20-25°C, relative humidity of 40-70%, a day cycle of 14 hours light and 10 hours dark, with a standard laboratory diet and free access to water. The Institutional Review Animal Care Committee approved the studies.

2.2 Anaesthesia and Euthanasia

The anaesthesia was induced with intramuscular ketamine (50 mg/kg) (Imalgene® 1000, Merial, Belgium) and xylazin (6 mg/kg) (XYL-M®, VMD, Belgium) and maintained with inhalational halothane (2%) and oxygen (1.5 l/m). At the end of the surgical procedures, except for the surgeries for induction of adhesions, the animals were sacrificed by an IV overdose of a mixture of embutamide, mebenzoniumiodide, tetracaine and dimethylformamide (0.5 ml) (T 61®, Hoechst Roussel Vet, Germany).

2.3 Surgical procedures

The animals were placed in supine position with the head next to the anaesthesia machine and the posterior legs next to the video-endoscopic tower. The abdomen was shaved from 3 cm below to the umbilicus up to 2 cm above to the costal line.

The surgeries were performed by one surgeon who stands at the left of the operation table and for an assistant who stands next to the head of the animal.

The xyphoides appendix was identified and an incision of 1 cm was performed in the abdominal wall, caudal to this landmark, for the introduction of the 1st trocar (10-12 mm) by open laparoscopy.

After the establishment of the CO_2 pneumoperitoneum using an insufflator (Thermoflator®, Karl Storz, Germany) with a flow rate of 5 l/m and an insufflation pressure of 6 mm of Hg, a 10-12 mm 0º endoscope connected to a single chip video camera and light source (Karl Storz, Germany), was introduced. Subsequently the 2nd trocar (5 mm) was inserted under direct laparoscopic vision at the level of the umbilicus to allow the introduction of the working instruments.

For some surgeries a 3rd and a 4th trocars were inserted. The place of insertion was defined according to the specific requirements of each surgery.

2.3.1 Surgical procedures for training in laparoscopy: Nephrectomy

This exercise is a model for dissection of major vessels and organs and includes the blunt and sharp dissection of the renal vessels, ureter and kidney using monopolar current when necessary. The instruments required are scissors, graspers, clip applier, clips, suction-irrigation probe and monopolar current.

For the left nephrectomy, the surgeon stands at the right side of the operation table and the animal is placed in right lateral position. The precise place of the introduction of the third trocar (5 mm) is chosen just after the visualisation of the left kidney in order to introduce the trocar perpendicular to the hilus. For the right nephrectomy the fourth trocar (5 mm) is introduced in the same way but with the surgeon at the left side of the operation table and the animal in left lateral position.

First both renal vessels, artery and vein, are dissected individually from the hilus up to the aorta and ligated with 3 clips, 1 for the artery and 1 for the vein proximally and 1 for the artery and vein together distally. Then they are cut. Secondly the ureter is dissected over some 6 cm from the hilus up to its intersection with the ovarian vessels, since this is an obvious anatomical landmark. Finally the kidney is dissected until its completed isolation, without surrounding fat, from the renal fossa.

2.3.2 Surgical procedures for induction of adhesions

For the experiments related with adhesion formation (Chapters 5, 6 and 7) the rabbits were placed in 45º Trendelenburg position and standardised opposing lesions of 2 cm^2 were performed in the uterine horns and in the pelvic sidewalls by either bipolar coagulation or CO_2 laser. Bipolar lesions were performed using a 5 mm forceps (Ethicon Endo-Surgery, Cincinnati, OH, USA) with a power of 10 watts (Force 30®, Valley Lab, Longbow Drive Boulder, CO, USA). CO_2 laser lesions (Sharplan 1040, Tel Aviv, Israel) were performed using a spot diameter of 1 mm with a power of 10 watts in the continuous super-pulse mode. Laser and bipolar lesions were used since the former is a lesion leaving a layer of some 100 µm of damaged cells only, whereas a bipolar lesion will induce necrosis up to at least a few mm. Since differences in healing between these lesions cannot be ruled out, the effect of pneumoperitoneum was investigated using both. The procedures took some 5-6 minutes and the pneumoperitoneum was maintained longer according to the design of each experiment. Taking into account the high exchange capacity of the peritoneum, a continuous flow rate through the abdominal cavity of 1 l/min was used to remove constantly any oxygen that could be diffused from the circulation. To achieve this, an 18-gauge catheter (Insyte-W®, Vialon®; Becton Dickinson, Spain) was inserted in between the first and the second trocars. At the end of the surgery the abdominal incisions were sutured with polyglactine 3-0 (Vicryl®, Ethicon®; Johnson and Johnson, Brussels, Belgium).

2.3.3 Surgical procedures for scoring of adhesions

For the experiments related with adhesion formation (Chapters 5, 6 and 7) second look laparoscopies were performed to score adhesions since we assumed that the laparoscopic evaluation might be more precise than a post mortem evaluation by laparotomy because of the magnification and because of the distended abdomen by the pneumoperitoneum. This, however, will have to be validated since it cannot be excluded that adhesions might be separated by the pneumoperitoneum.

2.4 Experimental design

The design of each experiment is described in the correspondent chapter. In all experiments block randomisation by days were used. The scoring system for the experiments related with training (Chapters 3 and 4) and with adhesion formation (Chapters 5, 6 and 7) were the following:

2.4.1 Scoring for duration and quality of surgery

Duration of surgery was scored in minutes from the beginning to the end of the dissection, i.e. after introduction of trocars and instruments.

Quality of surgery was scored using the scoring system described in Table 1. Quality of surgery score was defined as quality of dissection score minus total accidents score. Quality of dissection score was defined as the sum of the scores of dissection of renal vessels, ureter and kidney. Dissection of renal vessels, ureter and kidney were scored taking into account the quality of the procedure and the accidental lesions of these organs. Total accidents score were defined as the sum of bleeding score and lesions of adjacent organs (bowel, stomach, spleen, psoas muscle, etc.) score.

Table 1: QUALITY OF SURGERY SCORING SYSTEM

CATEGORY	DESCRIPTION	SCORE
DISSECTION OF RENAL VESSELS	No dissection Poor dissection Moderate dissection Perfect dissection	**0** **1** **2** **3**
DISSECTION OF URETER	No dissection Poor dissection Moderate dissection Perfect dissection	**0** **1** **2** **3**
DISSECTION OF KIDNEY	No dissection Poor dissection Moderate dissection Perfect dissection	**0** **1** **2** **3**
QUALITY OF DISSECTION	**DISSECTION OF RENAL VESSELS + URETER + KIDNEY**	**0 – 9**
BLEEDING	No bleeding Mild bleeding Heavy bleeding Mortal bleeding	**0** **1** **2** **3**
LESIONS OF ADJACENT ORGANS	No lesions Mild lesions Moderate lesions Severe lesions	**0** **1** **2** **3**
TOTAL ACCIDENTS	**BLEEDING + LESIONS OF ADJACENT ORGANS**	**0 – 6**
QUALITY OF SURGERY	**QUALITY OF DISSECTION – TOTAL ACCIDENTS**	**-6 – 9**

2.4.2 Scoring for adhesion formation

Since the lesions inflicted in either the right or the left sides were performed in the same way (laser or bipolar), scoring was done separately for right and left side, thus obtaining separate laser (L) and bipolar (B) lesions adhesion scores using the scoring system described in Table 1.

Laser total (L-total) adhesion score was obtained adding laser extent (L-extent), laser type (L-type) and laser tenacity (L-tenacity) adhesion scores. Bipolar total (B-total) adhesion score was obtained adding bipolar extent (B-extent), bipolar type (B-type) and bipolar tenacity (B-tenacity) adhesion scores. Extent, type, tenacity and total adhesion scores were defined as the sum of L-extent and B-extent, L-type and B-type, L-tenacity and B-tenacity and L-total and B-total, respectively.

Table 2: ADHESION FORMATION SCORING SYSTEM

CATEGORY	DESCRIPTION	SCORE
L-EXTENT or B-EXTENT	No adhesions 1-25% involved 26-50% involved 51-75% involved 76-100% involved	**0** **1** **2** **3** **4**
L-TYPE or B-TYPE	No adhesions Filmy Dense avascular Dense with capillaries Dense with larger vessels	**0** **1** **2** **3** **4**
L-TENACITY or B-TENACITY	No adhesions Essentially fall apart Required traction Required sharp dissection	**0** **1** **2** **3**
L-TOTAL or B-TOTAL	L-EXTENT + L-TYPE + L-TENACITY or B-EXTENT + B-TYPE + B-TENACITY	**0 – 11**
EXTENT	L-EXTENT + B-EXTENT	**0 – 8**
TYPE	L-TYPE +B-TYPE	**0 – 8**
TENACITY	L-TENACITY + B-TENACITY	**0 – 6**
TOTAL	L-TOTAL +B-TOTAL	**0 – 22**

2.5 Statistics

Statistical analysis were performed with the SAS System (80) using correlation (Spearman and Pearson) analysis, regression analysis (Proc LOGISTIC and Proc REG), Wilcoxon analysis and two way analysis of variance (General Linear Models, GLM) according to the requirements of each experiment.

CHAPTER 3

NEPHRECTOMY AND OTHER EXERCISES IN RABBITS: A NOVEL TRAINING MODEL FOR LAPAROSCOPIC SURGERY

3.1 Introduction

Training in laparoscopic surgery is important since laparoscopy is rapidly replacing laparotomy and since laparoscopic surgery requires specific skills. A variety of *in vitro* training models were developed in order to improve laparoscopic skills with promissory results (5-10). Training in animals has been proven efficient for increasing quality of surgery and decreasing operation time (4). In humans, as well, it has been proven that experience gained with training decreases operative accidents and duration of surgery (11-14)

Since the importance of training is obvious and since the lack of a reproducible *in vivo* training model, the objective of this work was the development of a model for training in laparoscopic surgery. This will permit the improvement of laparoscopic skills in order to standardise surgical techniques for the study of adhesion formation in the following experiments.

Therefore, we have developed the rabbit training model including a series of laparoscopic exercises which are described in detail in this chapter. In order to quantify the effect of training upon duration and quality of surgery, a prospective, randomised study was performed using the nephrectomy model.

3.2 Material and Methods

3.2.1 Animals and surgical procedures

Laparoscopic nephrectomies were performed in 80 rabbits as was described in Chapter 2. In addition, as a part of the training model, the following exercises were performed using a 3rd and 4th trocars in the left and right side of the lower abdomen:

3.2.1.1 Hysterectomy

The exercise is a model for dissection of organs and vessels and includes the blunt and sharp dissection of the ovarian vessels, ovaries, fallopian tubes, uterus, ureter, hypogastric and uterine vessels using monopolar current when necessary. The instruments required are scissors, dissector, suction-irrigation probe, needle holder, knot pusher, monopolar and bipolar current.

The animal is placed in 45° Trendelenburg position and the procedure is performed in one side until cutting the utero-sacro ligament. First the ovarian vessels are identified and dissected from the ovary up to the aorta, coagulated with bipolar current and cut. Secondly the ureter is identified and dissected from its intersection with the ovarian vessels up to its junction to the bladder. In the last part of its trajectory is identified its delicate relationship with the hypogastric and uterine vessels which are dissected, coagulated with bipolar current and cut. Then, the meso of the ovary, fallopian tube and uterine horn are cut as close as possible to the correspondent organ. The paracolpos is cut including the utero-sacro ligament. The same procedure is performed in the other side in the same way and finally the vagina is cut and the vaginal cuff is sutured with extra-corporeal or intra-corporeal knot tying.

3.2.1.2 Dissection of the mesenterica inferior

The exercise is a model for adhesiolysis using electro-surgery or laser-surgery. The difficulty is the dissection of major vessels over the entire length of the mesenterica inferior and of the recto-sigmoid. The instruments required are scissors, grasper, suction-irrigation probe, needle holder, knot pusher, monopolar and bipolar current and laser.

First, the normal adhesions between the sigmoid and the small bowel are dissected. Then, each branch that goes from the mesenterica inferior to the sigmoid wall is dissected individually, coagulated with monopolar or bipolar current and cut without injury to the bowel or the mesenterica inferior. The procedure can be performed with laser instead of electro-surgery. Finally two extra-corporeal knots are placed in the distal part of the bowel and two in the proximal part including the whole bowel and the mesenterica inferior vessels and then cut in between in order to remove the sigmoid and to control the ligature of the vessels.

3.2.1.3 Enterolysis

The exercise is a model for adhesiolysis and consists in the dissection of some 10 cm of the small bowel. The instruments required are scissors, grasper, suction-irrigation probe, monopolar and bipolar current and laser.

After the exposure of the bowel all vessels of the meso are dissected individually, coagulated with monopolar or bipolar and cut. The procedure can be performed with laser or with electro-surgery.

3.2.1.4 Model for salpingostomy

The exercise is performed in a loop of the small bowel. The instruments required are scissors, grasper, suction-irrigation probe, monopolar and bipolar current and laser.

A loop of some 6 cm of the small bowel is exposed. A knot is placed through the mesentery in one end simulating the uterine end. The other end is cut simulating the ampulla. Then a longitudinal incision is performed in the antimesenteric border and the intestinal contents are removed by grasping and by suction-irrigation. Coagulation with monopolar or bipolar current is performed when necessary.

3.2.1.5 Model for neosalpingostomy

The exercise is performed in the appendix. The instruments required are scissors, grasper, suction-irrigation probe, monopolar and bipolar current and laser.

A loop of some 6 cm of the appendix, which is a model for hydrosalpinx, is exposed including the stump, which simulates the closed fimbria. A knot is placed through the mesentery in the other end simulating the uterine end. With monopolar needle or with laser three radiary incisions are performed in the appendix stump and rinsed extensively with suction-irrigation. Finally coagulation with bipolar current with a power of only 5 watts is performed in order to obtain “flowering” of the distal part. This “flowering” can also be performed with laser with a power of only 5 watts in the continuous super-pulse mode and with the beam in a defocus position.

3.2.1.6 Removal of abdominal organs with endo-bags

The aim of the exercise is the management of endo-bags to remove abdominal organs including the morcellation when necessary. The instruments required are scissors, grasper, suction-irrigation probe and monopolar current.

Abdominal organs such as kidney, uterus, sigmoid, etc. are introduced in a finger glove (“endo-bag”) using 2 grasper and/or filling the bag with some fluid to keep it open.

When the organ is too big morcellation with scissors and monopolar current can be performed.

3.2.1.7 Suturing of the bladder

The exercise is a model for repair a defect of the bladder and suction of a cyst. The instruments required are scissors, grasper, suction-irrigation probe, monopolar current and laser.

A defect of some 3 cm is performed in an avascular part of the bladder with scissors or laser suctioning immediately its contents simulating the suction of a cyst. The bladder is sutured in 2 layers with single stitches and extra or intra-corporeal knots. Then, the bladder is filled with saline and methylene blue to assess if the suture is watertight.

3.2.1.8 Aorta and Cava dissection

The exercise is a model for dissection of major vessels. The instruments required are scissors, dissector, suction-irrigation probe, monopolar and bipolar current. Since this is a very difficult and potentially mortal exercise it should be performed at the end of the other exercises and only by more experienced surgeons.

The animal is placed in 45° Trendelenburg position and the aorta and vena cava are individualised removing the recto-sigmoid to the left side. First both vessels are dissected together starting at the level of the ovarian vessels until their bifurcation. Since the bifurcation of the cava is lower than the bifurcation of the aorta and since the vein is behind and at the left of the artery, like in humans, it is possible to identify a plane of cleavage in order to dissect aorta and cava separately. The small perforating branches are identified, coagulated and cut to avoid bleeding. Secondly the common iliac vessels are identified and dissected up to their bifurcation. A big branch that goes to the psoas muscle is coagulated and cut. Then artery and vein are separated. Finally the iliac intern and iliac extern arteries and veins are identified and dissected separately over some 2 cm. The fatty tissue and the lymphnodes over the entire length of the aorta and cava, common iliac and iliac intern and extern are dissected and removed.

3.2.1.9 Suturing exercises

The exercise is a model for stitching and knot tying in different positions and is performed in the abdominal wall of the animal. The instruments required are scissors, graspers, needle holder and knot pusher.

First longitudinal, transversal and diagonal defects of some 4 cm are performed in the anterior and lateral abdominal wall including the peritoneum and the muscle. Then the defects are sutured with singles or doubles stitching and extra-corporeal or intra-corporeal knot tying. Since these defects lead immediately to subcutaneous emphysema the exercise should be performed at the end and in a death animal.

3.2.2 Experimental design

In order to evaluate the effect of training 160 laparoscopic nephrectomies were performed by 8 surgeons. Each surgeon performed 20 nephrectomies.

In order to evaluate the effect of previous experience of the surgeons, the surgeries were performed by last year medical students without any experience in diagnostic or operative laparoscopy (n=6) and by gynaecologists who had finished their training in OBGYN, and who had a specific interest in endoscopic surgery but little experience and come to CHT in order to get additional training (n=2).

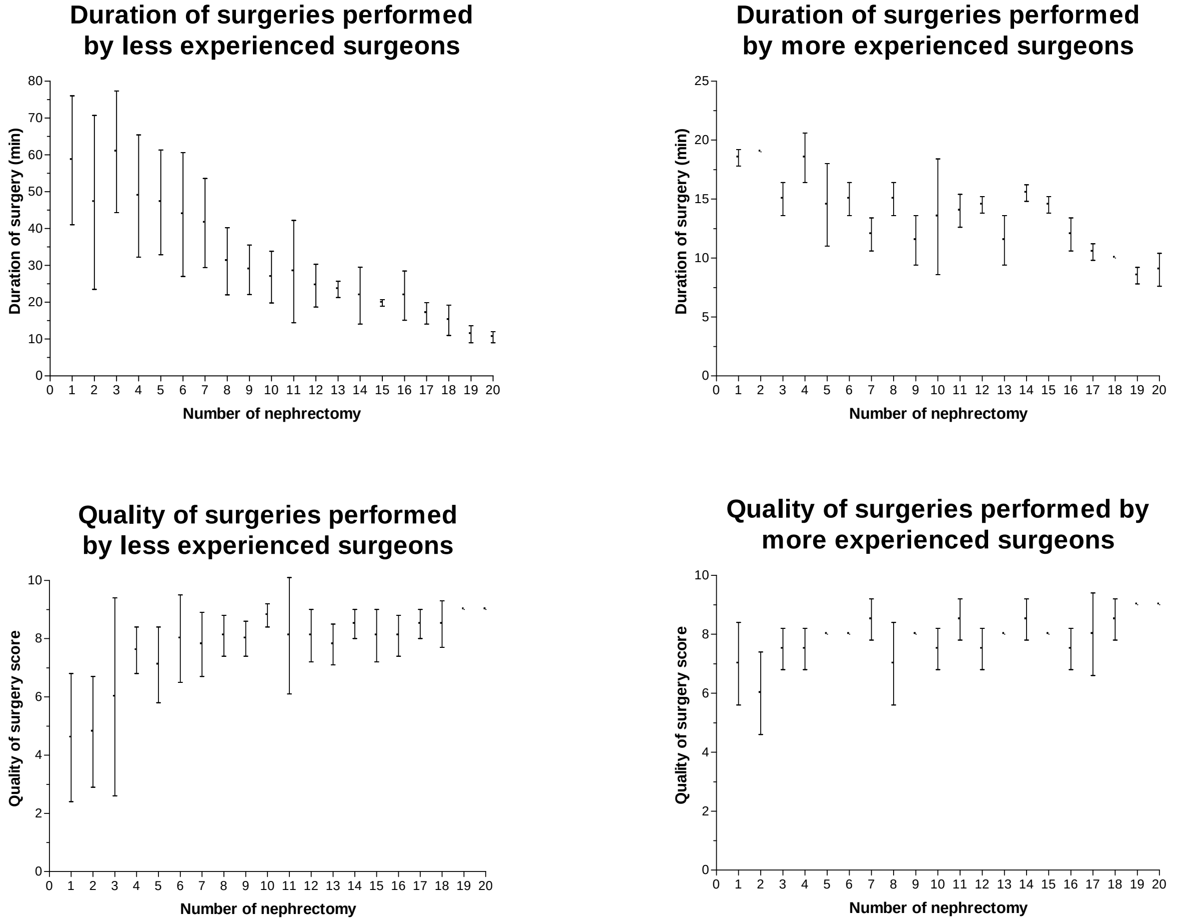
An independent investigator observed all procedures and subsequently scored duration and quality of surgery according to the scoring system described in Table 1 (Chapter 2). In order to show the effect of training, means and standard deviations (SD) were calculated sequentially for each surgery performed.

3.3 Results

A learning curve for both duration and quality of surgery was obvious in these experiments. Duration of surgery decreased whereas quality of surgery increased with training, assessed by the consecutive number of nephrectomies performed. These effects were observed for both less and more experienced surgeons.

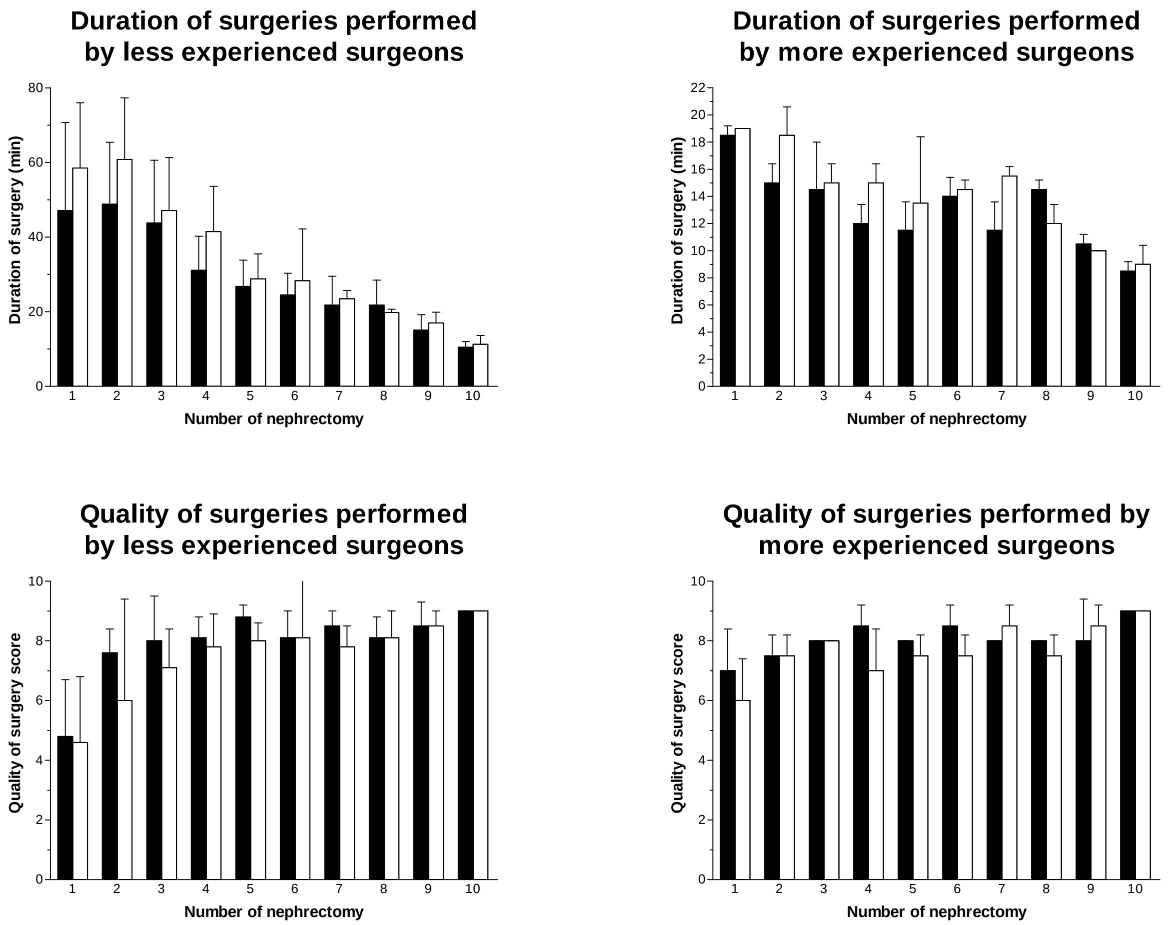
For less experienced surgeons duration of surgery decreased from some 60 minutes in the first interventions to some 10 minutes in the last interventions, whereas the quality of surgery scores increased from 4 to 9 respectively (Fig. 2). For more experienced surgeons duration of surgery decreased from some 20 minutes in the first interventions to some 10 minutes in the last interventions, whereas the quality of surgery scores increased from 6 to 9 respectively (Fig. 2). The numbers are too small for statistical evaluation but it seems obvious that less experienced surgeons initially have much longer operation times with lower quality than more experienced surgeon. After 20 nephrectomies these differences disappeared.

Fig. 2: Effect of training upon duration and quality of consecutive laparoscopic nephrectomies performed by less experienced and more experienced surgeons



Since right nephrectomy is more difficult than left nephrectomy they were evaluated separately. For both less experienced and more experienced surgeons, initially right nephrectomies were longer and with lower quality than left nephrectomies. After 10 left and 10 right nephrectomies these differences disappeared (Fig. 3).

Fig. 3: Effect of training upon duration and quality of left ■ and right □ laparoscopic nephrectomies performed by less experienced and more experienced surgeons



3.4 Discussion

For *in vivo* training in laparoscopy, rabbits seem to be a good model since they are relatively cheap and easy to handle. The surgeons can perform the anaesthesia without endotracheal intubation and, in general, no technical assistance is necessary. The main advantage is that laparoscopic exercises can be performed using conventional trocars and instruments.

The different exercises permit to work with either electro-surgery or with laser-surgery using several instruments such as scissors, needle holder, knot pusher, clip applier, suction-irrigation probe and different types of forceps. This allows to the trainees to improve their basic and advanced laparoscopic skills such as dissection, grasping, clipping, stitching, intra-corporeal and extra-corporeal knot tying.

The nephrectomy model combines the dissection of major vessels with fragile organs and since this model was designed in order to quantify the improvements in laparoscopic skills, i.e. duration and quality of surgery, it demands a special consideration. There are 2 ports to introduce the working instruments. The second port is at the level of the umbilicus and the third/forth (for left or right nephrectomy) port is perpendicular to the kidney' hilus. For the left nephrectomy the instrument through the second port is handed with the right hand and the instrument through the third trocar with the left hand. For the right nephrectomy is vice versa. The working instruments are scissors and forceps and, in a certain moment, a clip applier. The surgeons can use either the forceps or the scissors through either the second or the third/fourth ports according to their own abilities to manage the scissors with their right or their left hand. Whatever the position they choose, in some moment of the surgery the instrument through the second port is replaced by the clip applier for a few minutes. In this study and for the left nephrectomy, the right-handed surgeons used the scissors with the right hand through the second port and the forceps with the left hand through the third port in all cases. For the right nephrectomy, however, there was 2 different ways. Sometimes the surgeons used the scissors with the left hand through the second port and the forceps with the right hand through the fourth port in order to keep the relative position of instruments-organs as in the left side. On the contrary, sometimes they used the scissors with the right hand through the fourth port and the forceps with the left hand through the second port. Although they referred that they feel more confident with the latest, it is important to underline that this position is potentially more dangerous since sometimes the instrument through the fourth port is used to push away the kidney or the liver and since during the use of the clip applier there is not forceps available in the abdominal cavity to grasp a vessel that could be bleeding.

The right nephrectomy has 3 additional difficulties. First, the presence of the liver over the working area (at least over the upper part of the kidney) makes constant manipulation necessary. This is time consuming and dangerous due the potential massive bleeding. Second, the renal vessels are not parallel as in the left side but the artery is completely under the vein in its entire course except in the first 5 mm. Additionally the length of the vessels is shorter than in the left side. These elements make the working space smaller and closer to the aorta and cava with the consequent danger of bleeding. Third, the position of the instruments, as was mentioned previously, makes it more difficult for a right-handed person.

In the rabbit nephrectomy model, we observed and were able to quantify a clear learning curve for both duration and quality of surgery. The nephrectomy seems to be a good model for evaluating these variables because of the dissection of major vessels and since the surgeon has to adapt to the opposing requirements of going fast to reduce time but without having accidents as a heavy bleeding. At the beginning, most surgeons had severe surgical accidents including heavy bleeding and lesions of adjacent organs. The quality of dissection was poor, the duration of surgery longer and, since the differences mentioned above between the left and the right side, better scores were observed for left nephrectomies. With training all surgeon achieved shorter operation times with less accidents and better quality of dissection and the scores for left and right nephrectomies were more similar. Interestingly, the important differences observed between surgeons at the beginning of the training period disappeared gradually and were not anymore significant at the end.

These observations demonstrate what could be anticipating by common sense, i.e. that training improves the laparoscopic skills. This is useful for any surgical procedure and is especially important in the study of postlaparoscopic adhesion formation. This was already demonstrated since less experienced surgeons had more surgical accidents and more adhesions (4) emphasising the importance of standardisation of surgery.

Differences between experienced and non-experienced surgeons are preliminary data. Further experiments will be necessary to evaluate whether after 20 nephrectomies the non-experienced achieve the level of expertise as the experienced for other more difficult exercises.

In conclusion, the rabbit model is good for training in laparoscopic surgery since a variety of exercises with conventional instruments can be performed allowing the improvement of basic and advanced laparoscopic skills. In addition the rabbit is relatively cheap and easy to handle which is important for the institutions involved in endoscopy training.

CHAPTER 4

EFFECT OF THE DIAMETER OF THE ENDOSCOPE, SURGEONS' PREVIOUS EXPERIENCE AND TRAINING UPON DURATION AND QUALITY OF LAPAROSCOPIC SURGERY

4.1 Introduction

The assumption that the quality of the video-endoscopic image affects the quality of surgery is widely believed. Much effort was devoted to improve the quality of the image of the endoscopes and of the video systems. We witnessed the development of 3 chip cameras, electronic image enhancement systems such as digi-video, 3D camera's and fiberoptic and electronic endoscopes. Although the increased quality of the image is obvious, it still remains to be proven that this results in a better quality of surgery (81-83).

The quality of image is an important factor to take into account in the assessment of mini-endoscopy, which has been developed to decrease surgical trauma and to permit outpatient procedures. Since the quality of the image is less, it has been suggested that the skills required are higher than for traditional laparoscopy (84). Several studies failed however to demonstrate differences in the accuracy of the diagnosis with a 2 mm or a 10 mm endoscope (85-89). There still is no agreement for the optimal diameter of the endoscope when used for diagnostic procedures whereas the influence of the diameter and of the associated quality of the image upon the quality of the surgery has not been assessed properly.

In addition, and since most of our studies in adhesion formation will be performed in small animals such as rabbits and mice, the determination of the ideal diameter of the endoscope to obtain an optimal quality of surgery is very important.

Therefore, this prospective randomised trial was performed to assess the effect of the diameter of the endoscope upon duration and quality of surgery using the rabbit nephrectomy model. The effect the previous experience of the surgeon and the effect of the current training were used as confounding variables.

4.2 Materials and methods

4.2.1 Animals and surgical procedures

Laparoscopic nephrectomies were performed in 60 rabbits as was described in Chapter 2.

4.2.2 Experimental design

The study was carried out in 2 series of 60 nephrectomies.

To evaluate the effect of previous experience of the surgeon, one series was performed by an experienced and another series by an inexperienced surgeon. Both were gynaecologists with 3 years of experience in open surgery but with different exposure to laparoscopic surgery. The experienced surgeon had been exposed to more than 6 months of *in vitro* training, had performed more than 60 nephrectomies in rabbits in others experiments and a significant number of diagnostic and operative laparoscopies in humans. The inexperienced surgeon had 2 months of *in vitro* training and had been allowed to perform only 10 nephrectomies in rabbits, as his only exposure to laparoscopic surgery. The training of this surgeon had intentionally been kept to the strict minimum for two reasons. Firstly, an interim analysis of the results of the experienced surgeon had shown that the differences between small and larger endoscopes were so small, that very large series would be required to reach statistical significance. Secondly, since inexperienced surgeons had longer operating times and less quality, the hypothesis was formulated that an inexperienced surgeon would be a better model to demonstrate differences.

To evaluate the effect of the quality of the video-image the nephrectomies were carried out randomly by 0º endoscopes (Karl Storz, Germany) of different diameters. In the first series the experienced surgeon performed 20 nephrectomies using endoscopes of 10 and 5 mm (series 1A). Since the interim analysis did not show a difference in results, the subsequent 40 nephrectomies were performed using endoscopes of 10, 5, 4 and 2 mm (series 1B). In the second series the inexperienced surgeon performed 60 nephrectomies using endoscopes of 10, 4 and 2 mm (series 2).

Rabbits were randomised on a daily basis in blocks of 2 (series 1A), 4 (series 1B) and 3 animals (series 2). Since the nephrectomy of the right kidney seemed slightly more difficult, the left side was performed first and subsequently the right side was done using the same endoscope. In order to avoid the potential effect of the different relative position of the instruments for left and right nephrectomy randomisation was performed in animals, thus including 2 nephrectomies.

All procedures were continuously video-recorded and subsequently 2 independent observers scored blindly duration and quality of surgery as was described in Table 1 (Chapter 2).

4.2.3 Statistics

Statistical analysis was performed with the SAS System (80) using correlation (Spearman and Pearson) and regression analysis (Proc LOGISTIC and Proc REG).

4.3 Results

The quality of dissection of renal vessels, ureter and kidney were strongly intercorrelated (Spearman $P<0.0001$ for vessels-kidney, vessels-ureter and kidney-ureter). Therefore they were grouped into quality of dissection for further analysis.

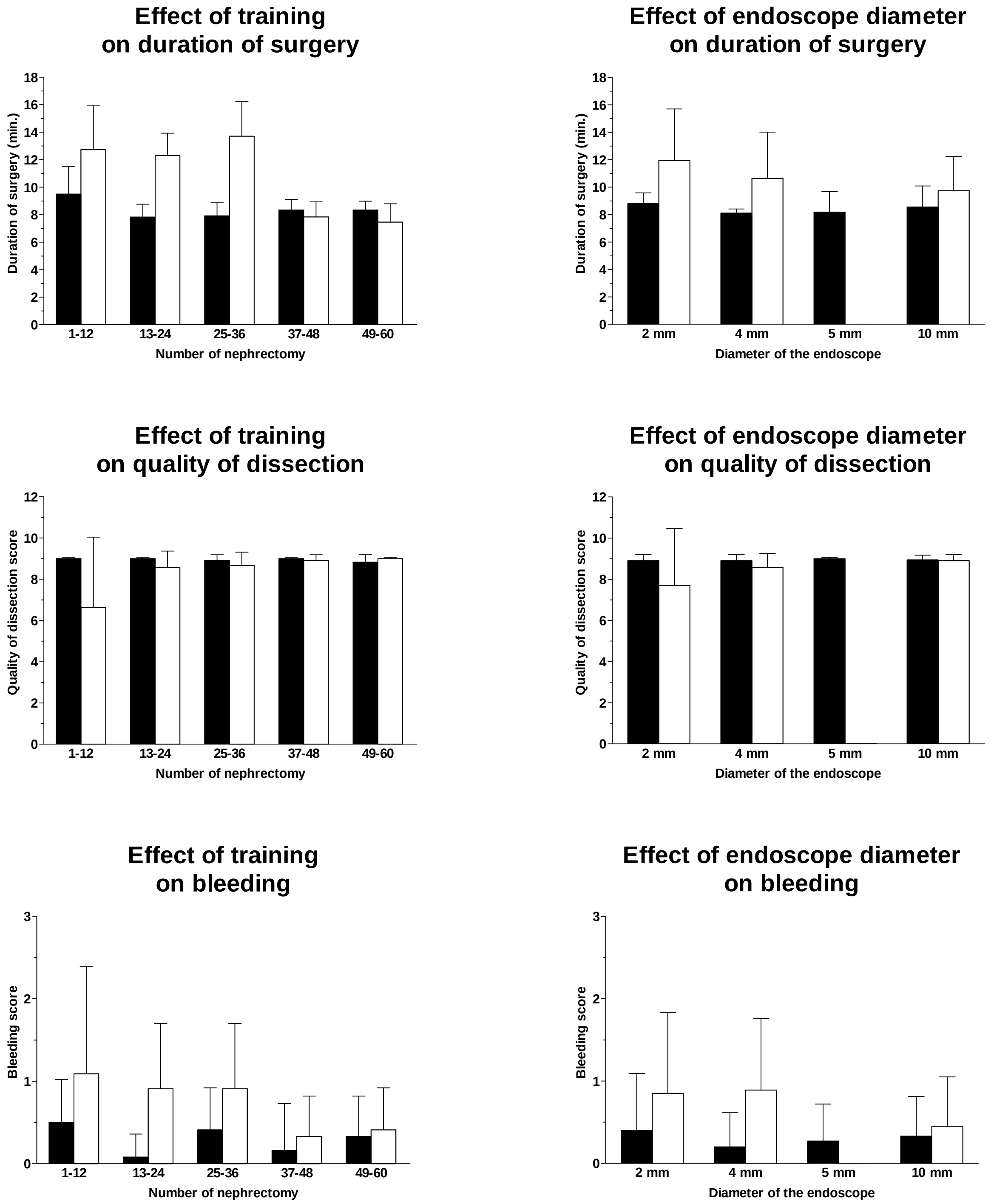
For the inexperienced surgeon, duration of surgery decreased (logistic regression) with the progressive gain in experience during the consecutives nephrectomies performed ($P=0.006$) and with a larger endoscope diameter ($P=0.015$) (Fig. 4).

The decrease in duration of surgery correlated (Spearman) with the increase in quality of dissection of renal vessels ($P=0.001$), ureter ($P= 0.012$) and kidney ($P=0.003$) and with the decrease in bleeding ($P=0.0002$) (Fig. 4).

By stepwise forward selection using regression analysis (Proc logistic) the duration of surgery was predicted simultaneously by the number of nephrectomy ($P=0.007$), endoscope diameter ($P=0.02$), quality of vessels dissection ($P=0.003$) and quality of ureter dissection ($P=0.03$).

For the experienced surgeon, duration of surgery only slightly decreased with consecutives nephrectomies (Fig. 3). In order to become statistically significant, logistic regression (Proc logistic) was necessary assessing simultaneously the effect of number of nephrectomy ($P=0.05$) and endoscope diameter ($P=0.06$) upon duration of surgery.

Fig. 4: Effect of training and endoscope diameter upon duration of surgery, quality of dissection and bleeding during consecutive nephrectomies performed by an experienced ■ and an inexperienced □ surgeon

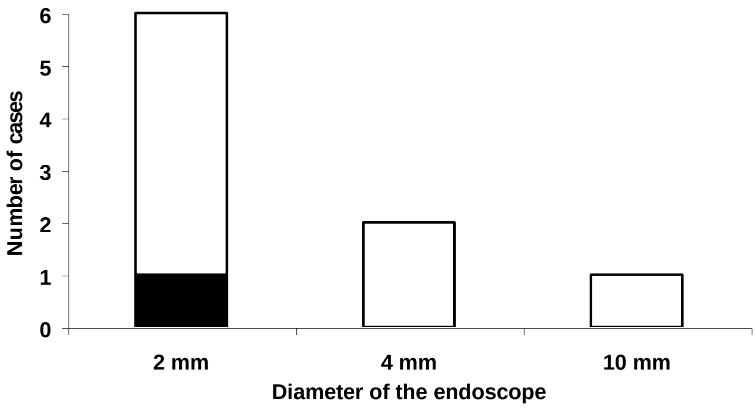


The effect of the endoscope diameter was investigated in more detail by regression analysis. For the experienced surgeon, no significant effect of the endoscope diameter could be demonstrated neither on the duration of surgery nor on quality of dissection or bleeding. For the inexperienced surgeon, the endoscope diameter affected simultaneously the duration of surgery (P=0.05), the quality of dissection (P=0.04) and the occurrence of bleeding

(P=0.08). In order to correct for the progressive gain in experience during the experiment it was found in one model that endoscope diameter and number of nephrectomy simultaneously affected duration of surgery (P=0.04 and P=0.001), quality of dissection (P=0.05 and P=0.002) and bleeding (P=NS and P=0.02) The same model was used to evaluate the differences between 10 and 4 mm endoscopes and between 4 and 2 mm endoscopes. For 10 and 4 mm endoscopes, endoscope diameter and number of nephrectomy simultaneously affected duration of surgery (P=NS and P=0.0001), quality of dissection (P=0.02 and P=NS) and bleeding (P=0.02 and P=NS). For 4 and 2 mm endoscopes, endoscope diameter and number of nephrectomy simultaneously affected duration of surgery (P=NS and P=0.0001), quality of dissection (P=NS and P=0.005) and bleeding (P=NS and 0.03).

Taking all data together in one regression model the endoscope diameter clearly affected simultaneously duration of surgery (P=0.04), quality of dissection (P=0.02) and bleeding (P=0.02).

Fig. 5: Heavy and mortal bleeding during laparoscopic nephrectomies performed by an experienced ■ and an inexperienced □ surgeon using endoscopes of different diameter



4.4 Discussion

To the best of our knowledge this is the first demonstration that the diameter of the endoscope affects the duration and the quality of surgery. This was clearly demonstrated for the inexperienced surgeon and for the total experiment. For the experienced surgeon the effect was statistically no significant but the number of animals required to pick up the small differences would probably be much larger. The diameter of the endoscope also affected the incidence of bleeding, which could be considered as an accident of surgery. Mortal accidents only occurred in 2 cases, both of them with a 2 mm endoscope and by the inexperienced surgeon. The inexperienced surgeon had also 6 cases of heavy bleeding, 1 with a 10 mm endoscope, 2 with a 4 mm endoscope and 3 with a 2 mm endoscope. Since heavy bleeding only occurred once in the experienced surgeon with a 2 mm endoscope, statistical significance could not be achieved. It is remarkable however that most accidents occurred with the smallest endoscope (Fig. 5).

The importance of these results for human surgery should no be underestimated. A decrease in the incidence of accidents is obviously important. However, to demonstrate a decrease of a phenomenon which only occurs rarely is very difficult since the sample size required to reach statistical significance is probably too high, e.g. to demonstrate the difference between 2% and 1% with a power of 80% and with a significance of 0.05 a prospective randomised trial of 5000 cases (2500 in both groups) is required. Our results therefore suggest that a better quality of the image will decrease the incidence of accidents, something that could be anticipated by common sense.

Our data confirm the well-known effect of training upon the learning curve (29,75-83). In the inexperienced surgeon, we found a considerable decrease in the duration of surgery and in the occurrence of bleeding and an increase in the quality of dissection after 20 rabbits. In the experienced surgeon, the effects of training upon the same variables were less important and did not reach statistical significance.

These data are important for the interpretation of mini-endoscopy results. Failing to show a difference between 10 and 2 mm endoscope in diagnostic procedures does not permit to conclude that both procedures are comparable. The reported series consisted of less than 100 cases with consequently a low power for these experiments (85-89). Moreover the surgeons were highly experienced and skilled. Our data suggest that for less experienced surgeons the difference between 10 and 2 mm could be much more important.

At the same time, this experiment permits to conclude that an endoscope of 10 mm is the ideal to perform the first series of studies in adhesion formation in the rabbit model and that further studies in mice could be performed with a 2 mm endoscope, especially once the surgeons involved are more experienced.

CHAPTER 5

ADHESION FORMATION INCREASES WITH INCREASING DURATION OF CO_2 PNEUMOPERITONEUM

5.1 Introduction

Laparoscopy is claimed to be less adhesiogenic than laparotomy, which was demonstrated in clinical (31) and in experimental (35-39) studies. Some studies failed, however, to demonstrate differences between the two different approaches (32-34). There are two factors to take into account in order to analyse this situation. First, it is universally assumed that the surgical trauma, which plays a key role in adhesion formation, is less during a laparoscopy than during a laparotomy. Second, laparoscopies are performed in a different environment that has to be considered. Indeed, CO_2, the most common gas used for creating the pneumoperitoneum for safety reasons, produces local effects in the peritoneum such as acidosis (41-43), desiccation (44), and adverse effects in the microcirculation (45,46), which could exert an influence in adhesion formation.

It was previously demonstrated that the experience of the surgeon and the duration of the laparoscopic surgery increase postoperative adhesion formation (4) but, because of the strong correlation, it was not possible to determine whereas this effect was due to the more severe surgical trauma in the less experienced surgeon or due to the duration of the surgery itself. To clarify this point a subsequent study has been performed with well-standardised known co-factors in adhesion formation. In this study pneumoperitoneum was maintained from 10 up to 60 minutes with humidified CO_2 at a flow rate of 20 l/m and an insufflation pressure of 10 cm of water. Again adhesion formation increased with duration of pneumoperitoneum (data not published). The same results were observed in mice when the duration of the pneumoperitoneum increased from 10 up to 120 minutes (40).

Therefore, this prospective, randomised trial in the rabbit model was performed to confirm the effect of duration of pneumoperitoneum, using no humidified CO_2 gas with a very low flow rate, and to evaluate the effect of CO_2 laser and bipolar lesions upon adhesion formation.

5.2 Material and Methods

5.2.1 Animals and surgical procedures

Standardised opposing lesions were performed in 20 rabbits by laparoscopy in order to induce adhesions. The lesions were performed randomly by bipolar coagulation in one side and by CO_2 laser in the other side. The pneumoperitoneum was maintained up to 10, 20, 30 or 60 minutes.

Second look laparoscopies were performed after seven days to score adhesion formation.

5.2.2 Experimental design

To evaluate the effect of duration of CO_2 pneumoperitoneum 4 groups of 5 animals were used (n=20). The pneumoperitoneum was maintained for 10, 20, 30 and 60 minutes in groups I, II, III and IV, respectively.

Block randomisation by days was used. Each block of 4 animals thus was operated during the same day. The same surgeon performed all surgeries during 5 consecutive days for the first and the second look respectively.

All second look laparoscopies were video-recorded and subsequently 2 independent observers scored adhesion formation blindly using the scoring system described in Table 2 (Chapter 2).

Since the lesions inflicted in either the right or the left side were performed in the same way (laser or bipolar), scoring was done separately for right and left side, thus obtaining separate laser (L) and a bipolar (B) lesions adhesion scores.

5.2.3 Statistics

Statistical analysis was performed with the SAS System (80) using Spearman correlation. The data are presented as mean ± SEM.

5.3 Results

All animals tolerated well the surgical procedure and neither operative nor postoperative complications were observed. Therefore, the adhesion scoring was performed in 5 animals in each group.

With increasing duration of CO_2 pneumoperitoneum the total adhesion score increased (P=0.0004) from 3.2 ± 1.9 after 10 minutes to 6.8 ± 2.4, 10.4 ± 2.6 and 14.4 ± 0.9 after 20, 30 and 60 minutes respectively, confirming the effect of duration of the pneumoperitoneum. The same effect was observed for each component of the adhesion score, i.e. extent, type and tenacity (Fig. 6).

Extent score increased (P=0.0004) from 1.0 ± 0.6 after 10 min. to 2.6 ± 0.8, 3.8 ± 0.9 and 5.8 ± 0.8 after 20, 30 and 60 minutes respectively. Type score increased (P=0.003) from 1.0 ± 0.6 after 10 min. to 2.0 ± 0.8, 3.4 ± 0.9 and 4.6 ± 0.9 after 20, 30 and 60 minutes respectively. Tenacity score increased (P=0.003) from 1.2 ± 0.7 after 10 min. to 2.2 ± 0.8, 3.2 ± 0.8 and 4.0 ± 0.3 after 20, 30 and 60 minutes respectively.

To evaluate the effect of different type of lesions, laser and bipolar lesions were scored separately. Increasing duration of the pneumoperitoneum increased L-total (P=0.0008), L-extent (P=0.0002), L-type (P=0.005) and L-tenacity (P=0.0008) adhesion scores as well as B-total (P=0.001), B-extent (P=0.01), B-type (P=0.01) and B-tenacity (P=0.01) adhesion scores (Table 3).

Fig. 6: Effect of 10, 20, 30 and 60 minutes of CO_2 pneumoperitoneum upon total, extent, type and tenacity adhesion formation following a laser and a bipolar opposing lesion. Mean scores ± SEM are indicated together with P values (Spearman).

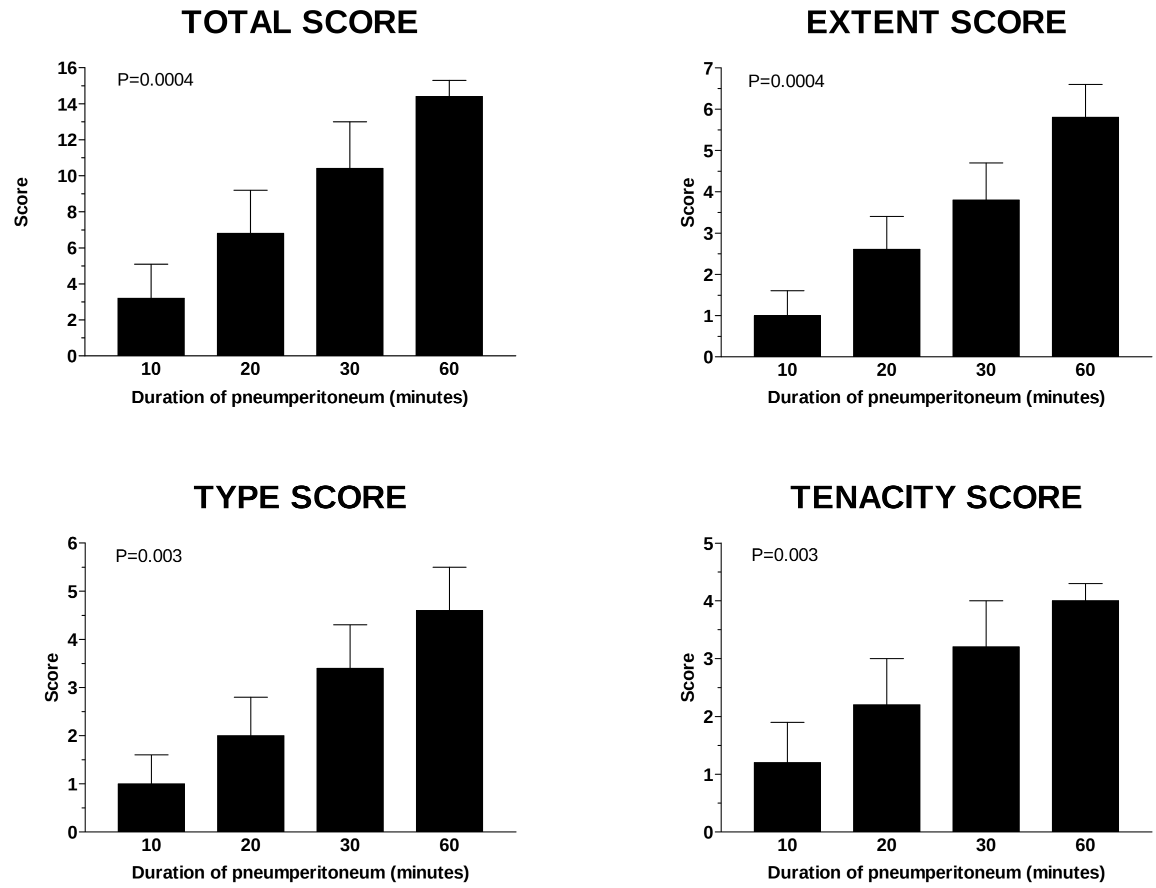


Table 3: L-extent, B-extent, L-type, B-type, L-tenacity, B-tenacity, L-total and B-total adhesion scores 7 days after the correspondent lesions using 100% CO_2 pneumoperitoneum for 10, (group I), 20 (group II), 30 (group III) and 60 (group IV) minutes. Mean ± SEM are indicated.

Type of lesion	Category	Groups			
		I	II	III	IV
Laser	L-extent	0.4 ± 0.2	0.8 ± 0.4	1.6 ± 0.5	3.2 ± 0.3
	L-type	0.4 ± 0.2	1.0 ± 0.6	1.8 ± 0.5	2.4 ± 0.5
	L-tenacity	0.6 ± 0.4	0.8 ± 0.4	1.6 ± 0.5	2.0 ± 0.0
	L-total	1.4 ± 0.8	2.6 ± 1.6	5.0 ± 1.5	7.6 ± 0.6
Bipolar	B-extent	0.6 ± 0.4	1.8 ± 0.4	2.2 ± 0.6	2.6 ± 0.5
	B-type	0.6 ± 0.4	1.0 ± 0.3	1.6 ± 0.5	2.2 ± 0.5
	B-tenacity	0.6 ± 0.4	1.4 ± 0.4	1.6 ± 0.4	2.0 ± 0.3
	B-total	1.8 ± 1.1	4.2 ± 1.1	5.4 ± 1.5	6.8 ± 0.6

5.4 Discussion

This study confirmed in the rabbit model the previous observation that postoperative adhesion formation increases with increasing duration of the pneumoperitoneum, at least up to 60 minutes. Since the mentioned study (unpublished data) used a relatively high flow rate and humidified CO_2 pneumoperitoneum the aim of this experiment was to evaluate whether the same effect is produced using non humidified CO_2 pneumoperitoneum with virtually no flow rate of less than 1 l/min, in order to minimise desiccation.

The increasing in adhesion formation was observed for extent, type and tenacity scores, which were strongly correlated. On the other hand this study was not able to find differences between laser and bipolar lesions but most specific studies are necessaries to determine the differences and the potential mechanisms of adhesion prevention after laser, bipolar or other type of lesions.

Although these data confirm the role of the CO_2 pneumoperitoneum in adhesion formation, the intrinsic mechanism remains unclear. It is well known that CO_2 produces local changes, such acidosis and desiccation, but it also produces, as any gas, hypoxemia due to the compression of the capillary flow in the superficial peritoneal layers.

Hypoxemia could be the main responsible since previous studies founded that adhesion formation increased with higher intra-abdominal pressures (47) and decreased with the addition of oxygen (48) in the rabbit model. To clarify this point it will be important to assess the effect of another gas, such helium, which does not produces acidosis.

In conclusion, this study confirmed that duration of CO_2 pneumoperitoneum is a co-factor in addition formation in the rabbit model. Therefore, this factor should be strictly standardised in further experiments and should no be underestimated in human surgery.

CHAPTER 6

HYPOXEMIA INDUCED BY CO_2 OR HELIUM PNEUMOPERITONEUM IS A CO-FACTOR IN ADHESION FORMATION IN RABBITS

6.1 Introduction

CO_2 pneumoperitoneum is the most common gas used for pneumoperitoneum for safety reasons because of its high solubility in water and exchange rate in the lungs. It was recently shown that CO_2 plays a role in peritoneal adhesion formation in rabbits (4) and in mice (40), which has been confirmed in this work (Chapter 5). CO_2 induces local changes such as intraperitoneal acidosis (41-43). In the absence of moistening, desiccation of mesothelial layers will occur (44), whereas the intraperitoneal pressure will induce adverse effects upon peritoneal microcirculation (45,46) possibly inducing hypoxemia. All these local effects of CO_2 could be involved in addition formation. Previous studies demonstrated that adhesion formation increase with higher intraperitoneal pressures (47) and decrease with the addition of oxygen (48) suggesting that hypoxemia, induced by the compression of the capillary flow in the peritoneal superficial layers during prolonged pneumoperitoneum, is a key adhesiogenic factor.

In order to confirm this hypothesis, this prospective, randomised trial in a rabbit model was designed using helium for pneumoperitoneum, knowing that this gas does not produce acidosis but produces, as any gas, hypoxemia.

6.2 Materials and methods

6.2.1 Animals and surgical procedures

Standardised opposing lesions were performed in 48 rabbits by laparoscopy in order to induce adhesions. The lesions were performed randomly by bipolar coagulation in one side and by CO_2 laser in the other side.

The pneumoperitoneum was created using 100% of CO_2 or helium or a mixture of 96% of CO_2 or helium with 4% of oxygen. This was achieved using 2 insufflators (Thermoflator®; Karl Storz, Tuttlingen, Germany), one for CO_2 or helium and one for oxygen. To obtain a homogeneous mixture, the output of both insufflators were mixed in a mixing chamber which was connected to a water valve to limit the insufflation pressure at 10 cm of water (90). Therefore a slightly higher insufflation pressure was used for both insufflators (8 mmHg), whereas 4% of oxygen was achieved using 24 l/min of CO_2 or helium and 1 l/min of oxygen. For reasons of standardization 25 l/min was used for pure CO_2 or helium knowing that all excess of gas will escape from the water valve. The pneumoperitoneum was maintained up to 10 or 45 minutes.

Second look laparoscopies were performed after seven days to score adhesion formation.

6.2.2 Experimental design

Six groups of 8 animals were used (n=48). In group I pneumoperitoneum was maintained for 10 minutes and in group II for 45 minutes using 100% of CO_2 and in group III for 45 minutes using 96% of CO_2 with 4% of oxygen. In group IV pneumoperitoneum was maintained for 10 minutes and in group V for 45 minutes using 100% of helium and in group VI for 45 minutes using 96% of helium with 4% of oxygen.

A 2 X 2 factorial design (groups I, II, IV and VI) was used to evaluate the effect of duration of pneumoperitoneum (10 and 45 minutes) and the effect of insufflation gas (CO_2 or helium) upon adhesion formation. Similarly a 2 X 2 factorial design (groups II, III, V and VI) was used to evaluate the effect of the addition of oxygen (100% of CO_2 or helium or 96% of CO_2 or helium with 4% of oxygen) and the effect of the insufflation gas (CO_2 or helium).

Block randomisation by days was used. Each block of six animals thus was operated during the same day. The same surgeon performed all surgeries during 8 consecutive days for the first and the second look respectively.

All second look laparoscopies were video-recorded and subsequently 2 independent observers scored adhesion formation blindly using the scoring system described in Table 2 (Chapter 2). Since the lesions inflicted in either the right or the left side were performed in the same way (laser or bipolar), scoring was done separately for right and left side, thus obtaining separate laser (L) and bipolar (B) lesions adhesion scores.

6.2.3 Statistics

Statistical analysis was performed with the SAS System (80) using Wilcoxon analysis and two ways analysis of variance. Since data were not normally distributed because of 0 scores, the general linear model (Proc GLM) was used instead of Anova. All data are presented as means ± SEM. The advantage of the 2 X 2 factorial design is that to achieve the same statistical precision, as with a one at a time approach, twice as many observations would have been needed. The power of the observed effect of duration of CO_2 and helium, and the effect of adding oxygen thus is comparable to experiments with 16 animals in each group. This increase in power of the factorial design is only valid when the effects of the 2 factors are additive, i.e. when no interaction between the 2 factors is present. The possibility to detect an interaction, i.e. a different effect of one factor at different levels of the other factor, can however also be considered an advantage of the factorial design, since this effect could otherwise easily be missed. When the number of observations is low, one should be aware that a positive interaction (with subsequent reduction of power to demonstrate the effect of the 2 factors) can be missed, especially when the between subject variability is high (91).

6.3 Results

Increasing the duration of pure CO_2 or helium pneumoperitoneum from 10 to 45 minutes, total adhesion score increased (Wilcoxon) from 4.6 ± 1.3 to 10.5 ± 1.8 for CO_2 (P=0.04) and from 3.1 ± 1.2 to 9.7 ± 1.8 for helium (P=0.02) confirming the effect of duration of pneumoperitoneum. For CO_2, extent increased from 1.7 ± 0.5 to 4.5 ± 0.9 (P=0.03), type from 1.3 ± 0.3 to 3.1 ± 0.5 (P=0.01) and tenacity from 1.6 ± 0.4 to 2.9 ± 0.6 (P=NS). For helium, extent increased from 1.1 ± 0.4 to 3.6 ± 0.6 (P=0.01), type from 1 ± 0.4 to 3.1 ± 0.6 (P=0.02) and tenacity from 1 ± 0.4 to 3 ± 0.6 (P=0.03). By two-way analysis of variance, the effect of duration of pneumoperitoneum was highly significant for total (P=0.0003), extent (P=0.0004), type (P=0.0004) and tenacity (P=0.004) scores, whereas no differences between CO_2 and helium and no interaction between duration of pneumoperitoneum and the type of gas used were found (Fig. 7).

Adding 4% of oxygen to CO_2 or helium pneumoperitoneum during 45 minutes, total adhesion score decreased (Wilcoxon) to 6 ± 1.8 for CO_2 (P=NS) and to 6.1 ± 0.9 for helium (P=NS). For CO_2, extent decreased to 2.4 ± 0.8 (P=NS), type to 1.8 ± 0.5 (P=NS) and tenacity to 1.8 ± 0.4 (P=NS). For helium, extent decreased to 2.1 ± 0.3 (P=NS), type to 1.9 ± 0.2 (P=NS) and tenacity to 2.1 ± 0.3 (P=NS). By two-way analysis of variance, the addition of oxygen was significant for total (P=0.02), extent (P=0.03), type (P=0.01) and tenacity (P=0.05) scores, whereas no differences between CO_2 and helium and no interaction between the addition of oxygen and the type of gas used were found (Fig. 7).

Laser and bipolar lesions were evaluated separately. By two-way analysis of variance, increasing duration of the pneumoperitoneum increased L-total (P=0.0001), L-extent (P=0.0001), L-type (P=0.0001) and L-tenacity (P=0.003) adhesion scores as well as B-total (P=0.05), B-extent (P=NS), B-type (P=NS) and B-tenacity (P=NS) adhesion scores (Table 4).

By two-way analysis of variance, the addition of oxygen decreased L-total (P=NS), L-extent (P=NS), L-type (P=NS) and L-tenacity (P=NS) adhesion scores as well as B-total (P=0.02), B-extent (P=0.04), B-type (P=0.01) and B-tenacity (P=0.05) adhesions scores (Table 4).

Laser lesions induced more adhesions than bipolar lesions as evidenced by higher total scores (P=0.0001), and higher scores for extent (P=0.0001), type (P=0.0001) and tenacity (P=0.0001).

Fig. 7: Effect of duration of CO_2 ■ and helium □ pneumoperitoneum (10 or 45 minutes) and of the addition of 4% of oxygen upon adhesion formation following a laser and a bipolar opposing lesions. Total, extent, type and tenacity means scores ± SEM are indicated together with P values (two way analysis of variance).

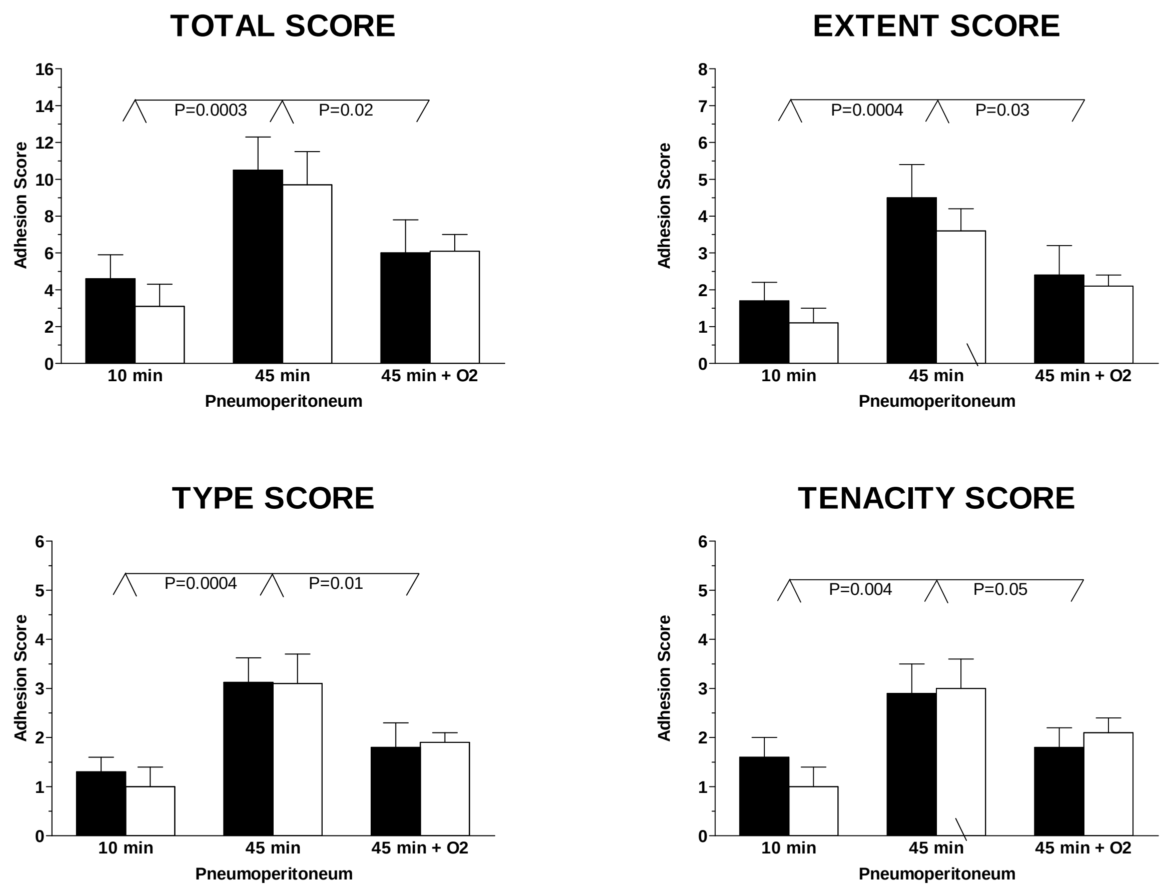


Table 4: L-extent, B-extent, L-type, B-type, L-tenacity, B-tenacity, L-total and B-total adhesions scores 7 days after the correspondent lesions using pure CO_2/helium pneumoperitoneum for 10 minutes (I/IV) and for 45 minutes (II/V) or 96% of CO_2/helium with 4% of oxygen for 45 min (III/VI). Mean ± SEM are indicated together with P values (two way analysis of variance).

Type of lesion	Category	Groups					
		I	II	III	IV	V	VI
Laser	L-extent	0.9 ± 0.5	2.9 ± 0.4 **	1.8 ± 0.6	0.8 ± 0.3	2.8 ± 0.4 **	2.0 ± 0.4
	L-type	0.5 ± 0.2	2.1 ± 0.3 **	1.4 ± 0.4	0.9 ± 0.3	2.1 ± 0.3 **	1.8 ± 0.3
	L-tenacity	0.8 ± 0.3	1.9 ± 0.3 **	1.3 ± 0.3	0.9 ± 0.3	2.0 ± 0.3 **	1.8 ± 0.2
	L-total	2.2 ± 1.1	6.9 ± 1.0 **	4.5 ± 1.3	2.6 ± 1.0	6.9 ± 1.0 **	5.6 ± 0.8
Bipolar	B-extent	0.8 ± 0.3	1.6 ± 0.5	0.6 ± 0.3 $^{\#}$	0.3 ± 0.2	0.8 ± 0.3	0.1 ± 0.1 $^{\#}$
	B-type	0.8 ± 0.3	1.0 ± 0.3	0.4 ± 0.1 $^{\#\#}$	0.1 ± 0.1	1.0 ± 0.3	0.1 ± 0.1 $^{\#\#}$
	B-tenacity	0.8 ± 0.3	1.0 ± 0.3	0.5 ± 0.2 $^{\#}$	0.1 ± 0.1	1.0 ± 0.3	0.3 ± 0.2 $^{\#}$
	B-total	2.4 ± 0.9	3.6 ± 1.0 *	1.5 ± 0.8 $^{\#}$	0.5 ± 0.5	2.8 ± 1.0 *	0.5 ± 0.5 $^{\#}$

I+IV versus II+V: $^{*}P<0.05$, $^{**}P<0.001$. II+V versus III+VI: $^{\#}P<0.05$, $^{\#\#}P<0.001$ (two way analysis of variance)

6.4 Discussion

To the best of our knowledge, this is the first study of postlaparoscopic adhesion formation using helium pneumoperitoneum. Helium was chosen as an alternative for CO_2 pneumoperitoneum because it is chemically, physiologically and pharmacologically inert, no explosive and since it does not produce hypercarbia and acidosis and the related hemodynamic and cardiopulmonary effects observed with CO_2 (91-97). Its lower solubility in water, however, carries a higher risk of embolization and an increased lethal effect of gas embolism (98,99).

Helium pneumoperitoneum was used in this experiment to evaluate the hypothesis that the hypoxemia, induced by compression of the capillary flow in the superficial peritoneal layers during prolonged pneumoperitoneum, is a cause of adhesion formation. It is logic to assume that increasing duration of pneumoperitoneum and higher intra-abdominal pressures limit the peritoneal capillary flow increasing hypoxemia and that the addition of oxygen could decrease hypoxemia. This assumption is consistent with our previous finding that duration of CO_2 pneumoperitoneum (4,40) and that higher intra-abdominal pressures (47) increase adhesions whereas the addition of oxygen decreases adhesion formation (48). This experiment confirmed the observations using CO_2 pneumoperitoneum and demonstrated the same effect using helium pneumoperitoneum whereas no differences were found between CO_2 and helium. All these data together confirm the hypothesis that hypoxemia is more important than acidosis in adhesion formation following prolonged pneumoperitoneum.

The effect of a low oxygen concentration in blood (hypoxemia) or in tissues (hypoxia) differs between tissues, which could be explained by a different micro-vascular flow reserves, oxidative capacity and metabolic need (100). Hypoxia alters the metabolism of endothelial cells as shown by vascular damage and increased vascular permeability (100), increased expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1) (101) and increased PMN adhesion to the vascular endothelium (102). At the same time, hypoxia decreases T-lymphocyte production of

IL-2, a key cytokine responsible for B-cell proliferation and inmunoglobulin secretion, and increases the release of TNF-α, IL-1 and IL-8 by human macrophages (101).

Hypoxia could modulate directly or indirectly, during pneumoperitoneum, the production of cytokines and growth factors by peritoneal mesothelial cells, macrophages and fibroblasts. Macrophages secrets cytokines and growth factors, such as IL-1, IL-4, IL-6, IL-10, TNF and TGF, which are involved in peritoneal wound healing and modulate the process that leads to adhesion formation (3). TGF-β1, TNF-α and IL-1β upregulate PAI-1 and downregulate t-PA, decreasing plasmin and thus inhibiting the lysis of fibrin (103). TGF-β decreases the expression of MMPs and increases the expression of TIMPs, thus decreasing matrix degradation and increasing fibrous adhesions (3). The effects of hypoxemia, however, have not been investigated as was done for mesothelial cells. Recently it was shown that human peritoneal mesothelial cells cultured under hypoxic conditions (2% oxygen) increase TGF-β1 mRNA and collagen III mRNA levels after 6 hours (104), TGF-β1 and TGF-β2 mRNA levels after 24 hours (105) and TIMP-1 mRNA levels, possibly via a TGF-β1 dependent mechanism (106). To interpret these data we should realise that in some experiments hypoxemia varies from 0% to 5% of oxygen. This could be important since it should be compared to the partial oxygen pressure in the abdominal cavity.

The effect of mesothelial hypoxemia, induced by the pneumoperitoneum during a laparoscopic surgery, upon vascular endothelial growth factor (VEGF) expression should be considered to explain the increase in adhesion formation. Indeed, hypoxia, together with other growth factors and cytokines, stimulates the production of VEGF by a variety of normal and transformed cell types (107). Hypoxia upregulates the production of VEGF by non-activated and by interferon-δ (IFNδ) and/or lipopolysaccharide (LPS) activated murine peritoneal macrophages (108). Increased levels of VEGF were detected, generally under hypoxic conditions, in ovarian hyperstimulation syndrome (109), ovarian neoplasm (110), endometriosis (111) and ascitis tumors (112) and during the normal cyclic changes in the female reproductive system (113). Furthermore, VEGF was found in peritoneal adhesions of women by inmunohistochemistry (114) and of men and women by ELISA (115) and it was shown that a policlonal rabbit antibody to VEGF limits adhesion formation after laparotomy in a murine model (116). These data indicate that VEGF has a role in the development of postoperative adhesions.

It is unclear at present why laser lesions induce more adhesions than bipolar lesions. We only can speculate that the larger denuded area might be important, but it should be investigated what the exact role of depth and amount of tissue necrosis is and whether this effect might be specific for hypoxemia induced adhesions.

In conclusion, this experiment confirms the key role of mesothelial hypoxemia in adhesion formation. Recent publications indicate that this could be mediated by a growth factor such as TGF-β or VEGF and most specific studies are currently been performed in order to clarify this point.

CHAPTER 7

EFFECT OF AN ANTIPROGESTIN UPON ADHESION FORMATION

7.1 Introduction

The effect of sexual hormones upon adhesion formation has been controversial.

Estrogens might have an important role in adhesion formation since hypoestrogenic status after treatment with GnRH-a or after oophorectomy is associated with decreasing adhesion formation (51-53) and since estrogen receptors were found in pelvic adhesion tissues (114). Other authors, however, found that estrogens decreased the murine MCP-1, affecting the macrophage recruitment and thus decreasing adhesion formation (58,59).

Progesterone has anti-inflammatory and immuno-suppresive effects (60-72) that could explain the reduction in postoperative adhesions after treatment with natural progesterone (73) or with MPA (74,75), a more potent synthetic progestagen. On the contrary, other studies failed to demonstrate this effect (74-79). However, the animal models, the type of lesion and the doses, via and time of administration of the progestagens were considerably different, which could affect the results.

Antiprogestins, such as mifepristone (RU486), with a marked noncompetitive antiestrogen effect (119,120) have been associated, as well with decreased postoperative adhesion formation (52).

All these contradictory data indicate that the sexual hormones could have an important role in the development of adhesions. Therefore, this prospective, randomised trial in the rabbit model has been performed in order to evaluate the effect of an antiprogestin upon adhesion formation in a dose-dependent and a in a time-dependent manner.

7.2 Material and Methods

7.2.1 Animals and surgical procedures

Standardised opposing lesions were performed in 48 rabbits by laparoscopy in 2 series of 30 rabbits in order to induce adhesions. The lesions were performed by CO_2 laser in both sides. The pneumoperitoneum was maintained up to 30 minutes.

Second look laparoscopies were performed after seven days to score adhesion formation.

7.2.2 Experimental design and medical treatment

The experiment was performed in 2 series of 30 animals (n=60) to evaluate the effect of the dose of antiprogestin (series 1) and the timing of administration of single and multiple doses of antiprogestin (series 2) upon adhesion formation.

In the first series 6 groups of 5 rabbits (n=30) received a treatment with antiprogestin (1ml, sc) immediately after the surgery. The dose of the antiprogestin used was as follows: Group A: 0mg/ml (only placebo), Group B: 0.02mg/ml, Group C: 0.066 mg/ml, Group D: 0.2 mg/ml, Group E: 0.66 mg/ml and Group F: 2 mg/ml.

In the second series 6 groups of 5 rabbits (n=30) received a treatment with antiprogestin (1ml per time, sc) at different time periods after surgery. The dose of the antiprogestins was 0.2mg/ml except in group I that received 0mg/ml (only placebo). The antiprogestin was given as follows. In order to evaluate timing of administration the drug was given once on day 0 (groups I and II), on day –1 (group V) and on day +1 (group VI). In order to evaluate the effect of multiple doses a single injection on day 0 (Group II) was compared to 1 or 2 additional treatment days, on day +1 (group III) and on days +1 and +2 (group IV) respectively.

The concentration of the antiprogestins for the second series was chosen after the analysis of the results of the first series where 0.2 mg/ml was a sub-optimal concentration.

Block randomisation by days was used in both series. Each block of six animals thus was operated during the same day by the same surgeon.

All second look laparoscopies were video-recorded and subsequently 2 independent observers scored adhesion formation blindly using the scoring system described in Table 2 (Chapter 2) but since the lesions inflicted in both right and left side were performed exclusively with laser the scoring was done separately in each side and then added.

7.2.3 Statistics

Statistical analysis was performed with the SAS System (80) using Wilcoxon analysis. Means ± SD are presented.

7.3 Results

In the first series, the effect of increasing concentration of antiprogestin was evaluated.

With increasing concentrations of antiprogestin total, extent, type and tenacity adhesions scores decreased. This effect was observed, even with the lowest dose of 0.002 mg/ml used (Fig. 8).

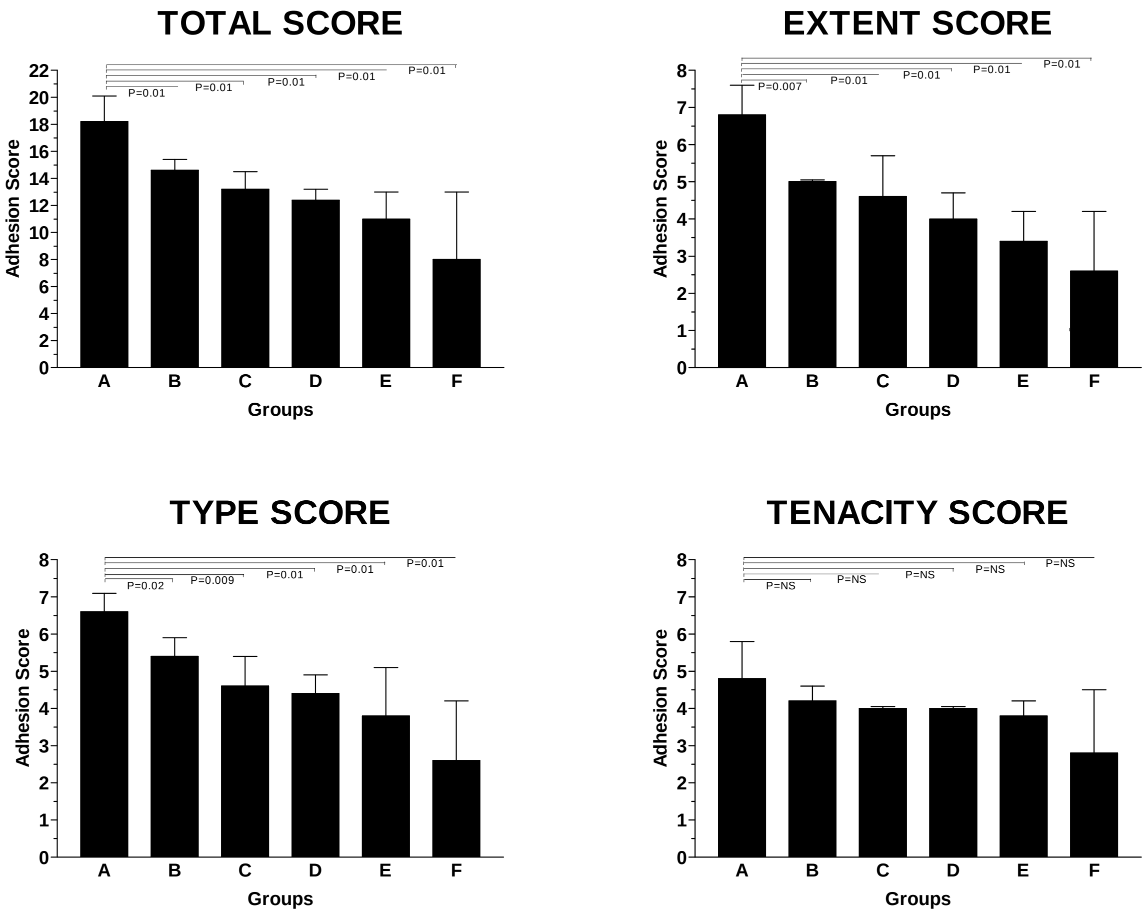
Total adhesion score decreased from 18.2 ± 1.9 in group A to 14.6 ± 0.8 in group B (P=0.01), to 13.2 ± 1.3 in group C (P=0.01), to 12.4 ± 0.8 in group D (P=0.01), to 11 ± 2 in group E (P=0.01) and to 8 ± 5 in group F (P=0.01).

Extent adhesion score decreased from 6.8 ± 0.8 in group A to 5 ± 0 in group B (P=0.007), to 4.6 ± 1.1 in group C (P=0.01), to 4 ± 0.7 in group D (P=0.01), to 3.4 ± 0.8 in group E (P=0.01) and to 2.6 ± 1.6 in group F (P=0.01).

Type adhesion score decreased from 6.6 ± 0.5 in group A to 5.4 ± 0.5 in group B (P=0.02), to 4.6 ± 0.8 in group C (P=0.009), to 4.4 ± 0.5 in group D (P=0.01), to 3.8 ± 1.3 in group E (P=0.01) and to 2.6 ± 1.6 in group F (P=0.01).

Tenacity adhesion score decreased from 4.8 ± 1 in group A to 4.2 ± 0.4 in group B (NS), to 4 ± 0 in group C (NS), to 4 ± 0 in group D (NS), to 3.8 ± 0.4 in group E (NS) and to 2.8 ± 1.7 in group F (NS).

Fig. 8 : Effect of an antiprogestin administered in different doses immediately after surgery upon total, extent, type and tenacity adhesions scores. Means ± SD are indicated together with P values (Wilcoxon).



In the second series of experiments, first the timing of a single administration of 0.2mg/ml of antiprogestins was evaluated.

Using a single administration, adhesions scores decreased in all groups, i.e. whether the drug was given on day –1 (group V), day 0 (group II) or day +1 (group VI). The decrease however was more pronounced when given on day 0 (Fig. 9).

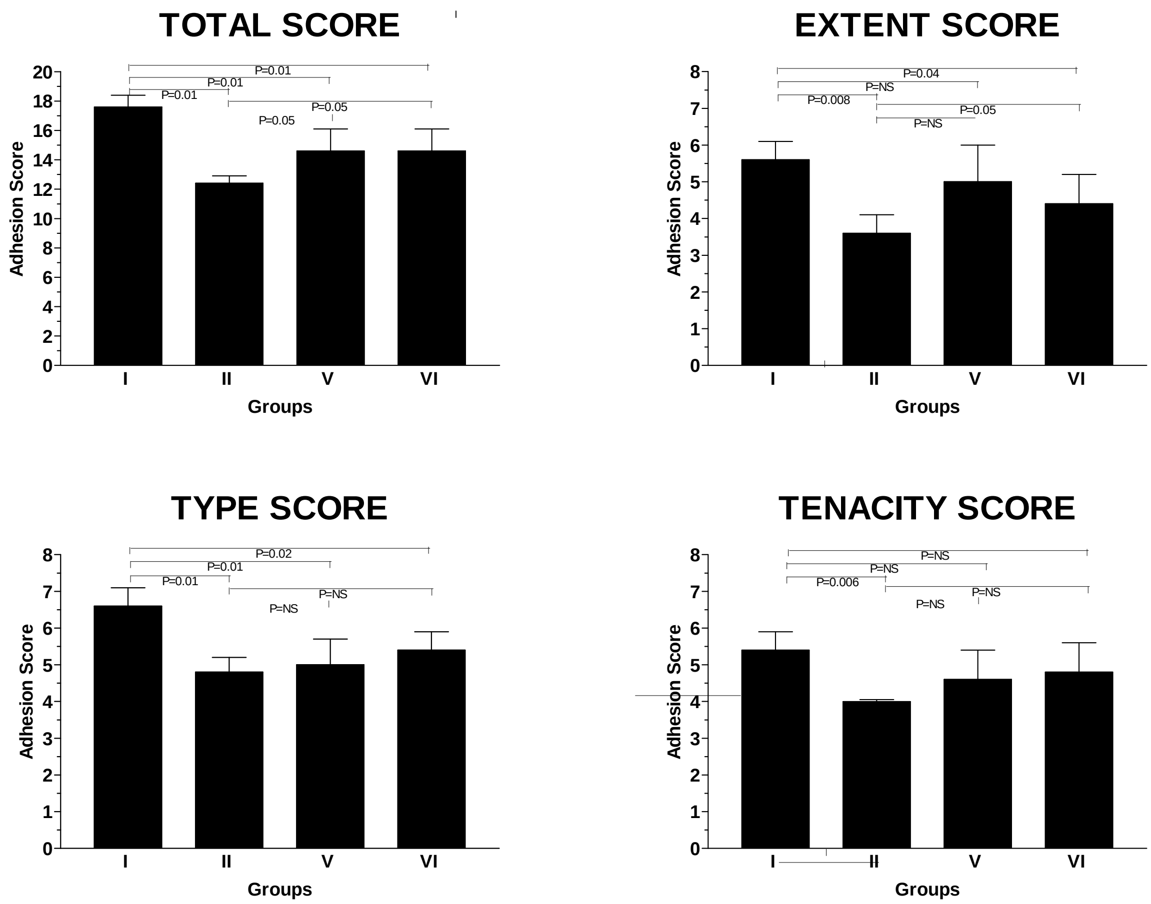
Total adhesion score decreased from 17.6 ± 0.8 in group I to 12.4 ± 0.5 in group II (P=0.01), to 14.6 ± 1.5 in group V (P=0.01) and to 14.6 ± 1.5 in group VI (P=0.01). In comparison with group II (day 0) the total score was higher in group V (P=0.05) and group VI (P=0.05).

Extent adhesion score decreased from 5.6 ± 0.5 in group I to 3.6 ± 0.5 in group II (P=0.008), to 5 ± 1 in group V (NS) and to 4.4 ± 0.8 in group VI (P=0.04). In comparison with group II (day 0) the extent score was higher in group VI (P=0.05) but not in group V.

Type adhesion score decreased from 6.6 ± 0.5 in group I to 4.8 ± 0.4 in group II (P=0.01), to 5 ± 0.7 in group V (P=0.01) and to 5.4 ± 0.5 in group VI (P=0.02). In comparison with group II (day 0) the type score was not significantly different neither in group V nor in group VI.

Tenacity adhesion score decreased from 5.4 ± 0.5 in group I to 4 ± 0 in group II (P=0.006), to 4.6 ± 0.8 in group V (NS) and to 4.8 ± 0.8 in group VI (NS). In comparison with group II (day 0) the tenacity score was not significantly different neither in group V nor in group VI.

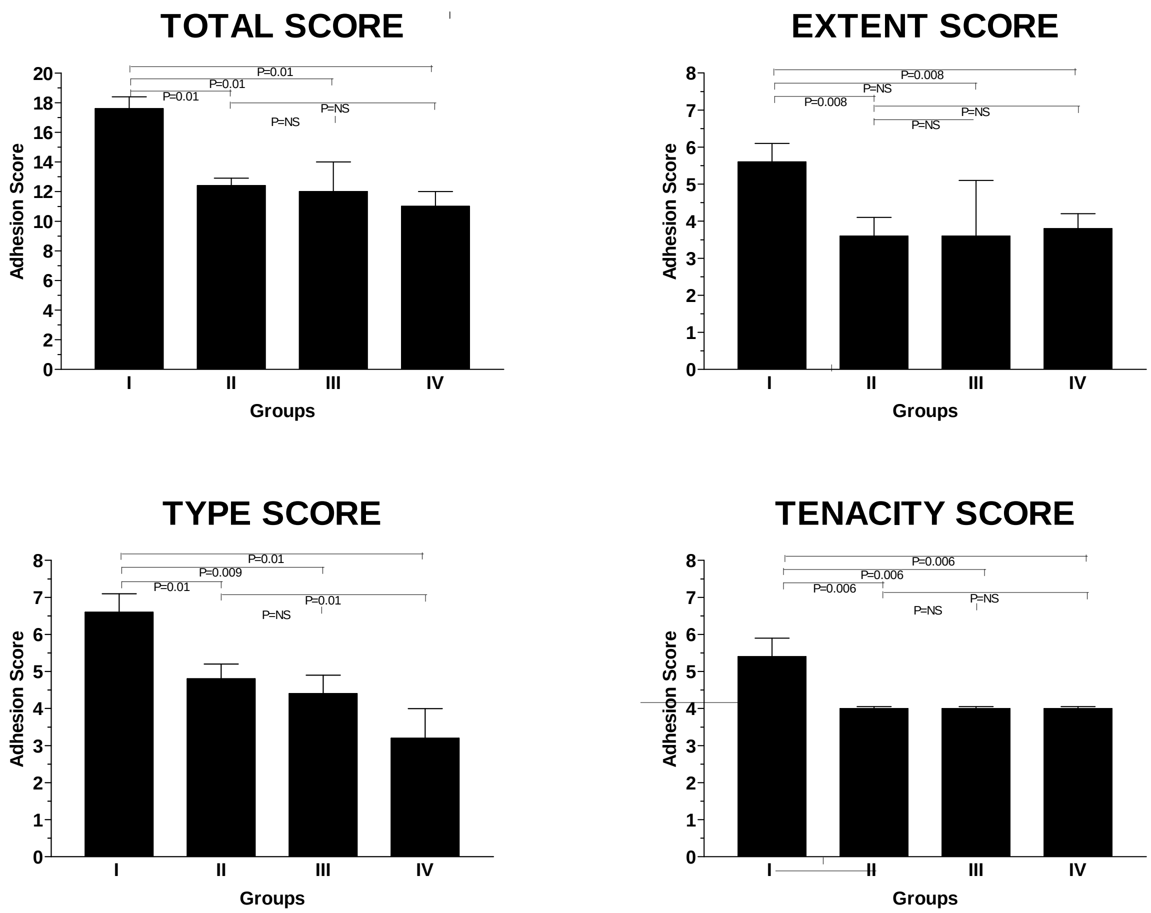
Fig. 9: Effect of a single dose of 0.2mg/ml of antiprogestin administered on day 0 (group II), day –1 (group V) or day +1 (group VI) upon extent, type and tenacity adhesions scores. Means ± SD are indicated together with P values (Wilcoxon).



In the second series of experiments, the effect of multiple doses of 0.2mg/ml of antiprogestins was also evaluated.

In comparison to treating on day 0 only (group II), drug administered on days 0 and 1 (group III) or on days 0, 1 and 2 (group IV), had slightly lower total, extent, type and tenacity adhesions scores, without reaching statistical significance with the exception of the type score in group IV (P=0.01) (Fig. 10).

Fig. 10: Effect of a single dose of 0.2mg/ml of antiprogestin on day 0 (group II) and multiples doses administered on days 0 and 1 (group III) or on days 0, 1 and 2 (group IV) upon extent, type and tenacity adhesions scores. Means ± SD are indicated together with P values (Wilcoxon).



7.4 Discussion

These data clearly demonstrated that antiprogestins reduce adhesion formation in a dose-dependent and in a time-dependent manner.

Total, extent, type and tenacity adhesion formation decreased progressively with increasing concentration of the antiprogestins at least up to 2 mg/ml. Although our data does not permit to determine the maximal effect since we did not use a concentration superior to the mentioned above, it is important to emphasise that this effect was observed even with the minimal concentration used (0.02 mg/ml).

When 0.2 mg/ml of antiprogestin was given at different times we observed a decrease in adhesion formation regardless the number of doses and the time of administration.

Total adhesion scores were higher when the treatment was given either on day +1 or day –1 instead of in day 0, indicating that a difference of only one day, before or after, is enough to limit the action of the antiprogestin.

On the other hand, additional treatment on days 1 and 2 did not reduce adhesion formation more than a single dose in day 0, indicating a very low sum of effect.

All these together strongly indicate that the best effect is achieved when the treatment is given as close as possible to the surgical trauma.

Although the beneficial effect of the antiprogestin observed in this study is obvious, similar to the found with RU486 (52) and previously for our group with onapristone (unpublished data), the intrinsic mechanism remains unclear and demand further experiments.

CHAPTER 8

GENERAL DISCUSSION

The importance of training for the improvement of surgical skills has been well established in a variety of studies (4-14). This is especially important in laparoscopy since it demands different skills than laparotomy. Although training in endotrainers and in animals in workshops is useful, it is obvious that in 1 day only techniques can be taught without really training the skills. To the best of our knowledge this is the first report of the development of a dissection model and the necessary introduction of a cheaper model, i.e. the rabbit. With this model trainees learn to use several instruments during electro-surgery or laser-surgery. Special consideration has to be given to the nephrectomy, which is a model for dissection of major vessels. It permits to quantify duration and quality of surgery, since the surgeon has to adapt to the opposing requirements of going fast to save time, but without having accidents such as a heavy bleeding. Dissection together with stitching and knot tying are the most important skills necessary to perform a laparoscopic surgery. Although stitching and knot tying skills can be acquired in the rabbit training model, it is obviously easier to quantify them in endotrainers. For this reason in addition to the rabbit model we emphasised in stitching and knot tying exercises in endotrainers. A prospective study is actually being performed in order to evaluate the effect of training upon duration and quality of stitching in different angles and of intra-corporeal knot tying, together with nephrectomy. This study is performed by last year medical students who wish to start the specialization in gynaecology. From the results obtained so far, it is clear that a typical learning curve is seen for knot tying (7), stitching and nephrectomy. It will have to be evaluated to what extent this training will affect other training exercises since this will be the first indication that training could reduce operation time in the hospital. The final goal of this project is to reduce the duration of the surgical procedures in operation theatre in order to reduce costs and to improve patient care.

Another advantage of this training model was that it permitted the standardisation of surgical techniques. This is crucial in the study of adhesion formation since it was demonstrated that less experienced surgeons achieved longer operation times and had more adhesions (4).

In addition to the rabbit training model we standardised the uterine rabbit model for the study of postlaparoscopic adhesion formation. In order to avoid any operative or postoperative complication the surgeries were performed under strict aseptic conditions. Open laparoscopy technique was used since in preliminary studies we found a high incidence of bowel injury with the Veress needle insertion, due to the very thin abdominal wall. An insufflation pressure of 6 mm of Hg was used since after 60 minutes anaesthetic problems were found with higher insufflation pressures in these non-intubated rabbits. The animals were placed in 45° Trendelenburg position and the bladder was emptied in order to obtain a good exposition of the uterus and to use only one instrument. Therefore we used only 2 laparoscopic ports instead of the traditional 3. The duration and the type of lesions and the surgeons' skills, as mentioned above, were also standardised to avoid well-known co-factors in adhesion formation such as bleeding, excessive surgical trauma and longer procedures. On the other hand we found a lot of zero scores in some experiments indicating that more severe and larger lesions should be performed. Other co-factors in adhesion formation, such as desiccation and the estrogenic status of the animal, should also be well standardised.

Laparoscopy is rapidly replacing laparotomy in both general and gynaecological surgery. Therefore evaluation of postlaparoscopic adhesion formation is especially crucial, since our data demonstrate that CO_2 pneumoperitoneum is a co-factor. These data confirmed that postoperative adhesion formation increase with increasing duration of pneumoperitoneum and decrease with the addition of oxygen. Since the same effect was observed using either CO_2 or helium pneumoperitoneum the effect of pH changes is probably less important. This strongly suggests that hypoxemia of the superficial mesothelial layers induced by the pneumoperitoneum is a key factor in adhesion formation, moreover since adhesions increase with higher intra-abdominal pressures (47).

These observations could lead to novel means of adhesions prevention in the human. With this aim a prospective, randomised trial will be performed in humans to evaluate the effect of the pneumoperitoneum with a small amount of oxygen upon postoperative pain. This study will be carried out using a recently developed insufflator (Thermoflator Plus®, Karl Storz, Germany), which permits to add very precise amount of oxygen to the CO_2 pneumoperitoneum. In addition, and taking into account the availability of transgenic mice with over or under expression of VEGF, a variety of experimental studies will be performed in order to clarify the role of VEGF in postoperative adhesion formation.

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