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The effect of training and duration of surgery on adhesion formation in the rabbit model

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In order to evaluate the effect of training upon postoperative adhesions, standard bipolar and mechanical, non-opposing injuries were performed in the uterine horns and side walls of 52 mature female rabbits using a conventional three-puncture laparoscopy, by an endoscopic surgeon with limited experience. An additional injury, either bipolar or mechanical or both, was performed in the retro-uterine space. With experience, the duration of surgery decreased progressively from 12 ± 2 to 8 ± 1 min in the first and last 10 animals respectively. The amount of perioperative bleeding was not affected by experience. With experience the postoperative adhesions decreased in extent ($P = 0.0001$), tenacity ($P = 0.004$), type ($P = 0.002$) and inflammation ($P = 0.003$) and for total score ($P = 0.0002$). These changes were correlated with the briefer duration of surgery but not with the amount of perioperative bleeding. The strong correlations of adhesion scores in the pouch of Douglas, and around both uterine horns confirmed the importance of the inter-animal variability in making adhesions. By logistic regression, the adhesions in the pouch of Douglas were explained simultaneously by the adhesions on the uterine horns ($P = 0.0004$, thus correcting for inter-animal variability) by the amount of bleeding ($P = 0.01$) and the duration of surgery ($P = 0.05$). No major differences were found in adhesions following a mechanical or a bipolar injury or following such a lesion in the pouch of Douglas or at the uterine horns. In conclusion, experience, expressed by the duration of surgery and to a lesser extent perioperative bleeding, is a major co-factor in postoperative adhesions, suggesting that duration of surgery should be strictly standardized in endoscopic adhesion studies. The important inter-animal variability can be circumvented by using a standard control lesion, making each animal its own control.

Key words: adhesions/endoscopy/laparoscopy/rabbit/training

Introduction

Postoperative adhesion formation remains a major clinical problem. Following a single laparotomy, adhesions occur in some 53–91% of patients (Doody *et al.*, 1989) and following multiple laparotomies >90% of patients have adhesions. Postoperative adhesions are the most frequent cause of bowel obstruction (Ellis, 1982) and pelvic adhesions are a major cause of infertility (Drake and Grunert, 1980). Whether they are caused by infection, endometriosis or surgery, there is still no adequate prevention or treatment. The mechanism of postoperative adhesion formation is multifactorial. Peritoneal injury and subsequent inflammatory reaction results in fibrin deposition and fibrinolysis. The peritoneal repair process, which is still incompletely understood, includes a crucial role for macrophages, the intraperitoneal immune system, angioneogenesis, fibroblast growth and collagen deposition. Prevention of adhesions has been attempted by keeping the injured peritoneal surfaces separated, either by using highly viscous fluids, such as hyscon, mechanically, or by modulating the inflammatory reaction using a wide range of products, such as glucocorticoids, non-steroidal anti-inflammatory drugs, calcium channel blockers etc. (for review diZerega and Rogers, 1992; diZerega, 1994).

Laparoscopic surgery has been claimed to be less adhesiogenic than a laparotomy (Luciano, 1990). This has, however, never been demonstrated conclusively, at least partly because of ethical constraints in man, and also because most studies aim to show the benefits of laparoscopic surgery, such as lower postoperative morbidity and pain. The issue of adhesion formation following laparoscopy has been addressed in a number of animal studies, comparing laparotomy and laparoscopy, but the conclusion is still equivocal. In rats Filmar *et al.* (1987) found no statistically significant differences between laparoscopy and laparotomy, after mechanical injury with scissors. In rabbits Luciano *et al.* (1989), compared adhesion formation after a laser injury in a laparotomy and laparoscopy group. They concluded that after a laparotomy more postoperative adhesions were found together with de-novo adhesions. Marana *et al.* (1994) found no difference in adhesion formation following laparotomy or laparoscopy for ovarian conservative surgery in rabbits. Also Jorgensen *et al.* (1995) found no differences in adhesions after a standardized mechanical lesion in rabbits, although laparoscopy was associated with a lower incidence of wound adhesions.

Besides well-known adhesiogenic factors such as postoperative infection and bleeding, the specific effects of training and experience, and of the duration of surgery, often mentioned as possibly important co-factors, have not yet been specifically investigated. Also, the inter-animal variability has not yet been taken into account, although clinical experience suggests that some patients are more adhesion-prone than others. We

Table I. Classification of intraperitoneal adhesions by laparoscopy

	Score				
	0	1	2	3	4
Type	absent	filmy	dense	vascular	
Extent (%)		0–25	25–50	50–75	75–100
Genital coverage	absent	<50%	>50%		
Wall coverage	absent	present			
Gastrointestinal coverage	absent	present			
Urinary tract coverage	absent	present			
Total score (sum)					

therefore wanted to investigate these factors in a rabbit model using conventional laparoscopy.

Materials and methods

Experimental design

The experiment was designed to evaluate the effect of training upon postoperative adhesion formation and to evaluate a method to correct for the inter-animal variability in adhesion formation. The procedures were performed by a qualified gynecologist (J.O.) with a wide experience in diagnostic laparoscopies but without any experience in operative laparoscopic procedures in humans or in animal models. The effect of training was evaluated by the duration of surgery (in min) and the amount of bleeding (scored as absent, mild, moderate or severe). The possibility to correct for inter-animal variability was evaluated by inflicting a control lesion to the uterine horn and side wall, and a (test) lesion to the retro-uterine space. The retro-uterine space was chosen to mimic pelvic surgery in the human. It was anticipated that the retro-uterine space and the oviducts would be sufficiently separated to permit subsequent, specific experiments modulating adhesion formation in the retro-uterine space, without affecting adhesion formation around the uterine horn, which thus could serve as a control lesion.

All animals received two control lesions, consisting of a mechanical excision with scissors of 2 cm^2 of peritoneum at the level of the ovary, and bipolar coagulation for 3 s at 10 W over an area of 2 cm^2 of the uterine horn surface (Force II, Valleylab®; Zaventem, Belgium). In addition a test lesion was made in the right and left lower parts of the mesometrium in the pouch of Douglas, consisting of either a mechanical lesion (2 cm^2 of peritoneum removed by sharp dissection, $n = 18$, group I), or a bipolar lesion (coagulation of 2 cm^2, $n = 15$, group II), or a combination of both lesions ($n = 17$, group III). The sequence of groups I, II and III was determined by randomization tables with blocks of three animals. Each block of three animals was operated on in a single session during the same day. Seven days later adhesions were scored at a second look laparoscopy under general anaesthesia. All the procedures and second look laparoscopies were videotaped, and subsequently blindly scored for blood loss and adhesions by two independent investigators. Adhesions were scored according to Fiedler *et al*. (1996) in a scoring system designed to score the total amount of adhesions after several weeks, taking into account extent (0 to 4 points), type (0 to 4 points), tenacity (0 to 3 points) and inflammation (0 to 4 points). In order to score adhesions after only 7 days and to score each individual lesion (pouch of Douglas, left and right uterine horn) and also to allow us to distinguish between the extent of adhesions at the site of injury and in the surrounding area, the modified scoring system described in Table I was used. Tenacity and inflammation were omitted from this scoring system, since scoring was done after 7 days, whereas the weighting given to the extent of the adhesions was increased to 6 points. The Fiedler score will be called adhesion score 1 (scoring from 0 to 15) and our scoring system adhesion score 2 (scoring from 0 to 12 in three locations). As expected, adhesion score 1 and adhesion score 2 were strongly correlated (Pearson's $R = 0.89$, $n = 50$, $P = 0.001$).

Animals and surgical procedures

Mature, female, New Zealand white rabbits ($n = 52$) weighing between 2100 and 2700 g were used. The study was approved by the Institutional Review Animal Care Committee, and the animals were housed at the Centre for Laboratory Animal Care at the Catholic University of Leuven (K.U. Leuven). Two animals died (numbers 2 and 14) and were obviously excluded from the study.

After premedication with intramuscular ketamine (50 mg/kg, Ketalin® p.i; Apharmo, The Nederlands) and xylazin hydrochloride (0.3 mg/kg Rompum® 2%; Bayer, Belgium), anaesthesia was maintained with inhalational halothane at 50% (Fluothane®; Zeneca, Belgium) and 1 litre/min of oxygen. The animals were placed in a supine position, the abdomen shaved and disinfected with polyvidone iodine (iso-Betadine®; Asta Medica, Belgium). The surgery was done under strict aseptic conditions and no perioperative antibiotics were administered. A standard three-puncture laparoscopy was performed using conventional equipment. At laparoscopy, a 10 mm trocar (Ethicon®; Endosurgery, Belgium) was placed caudal to the sternum. Pneumoperitoneum was initiated with a CO_2 flow rate of 1 litre/min (Thermoflator®; Karl Storz, Tüttlingen, Germany). The intra-abdominal pressure was 5 mmHg. A 10 mm 0° scope connected to a single chip video camera and light source was used (Karl Storz). Under direct vision two 5 mm trocars were introduced in the left and right flanks, through which atraumatic grasping forceps or scissors were used. To standardize the procedure, neither irrigation nor aspiration of blood was performed. After the procedure the abdominal incisions were sutured with 3-0 Vicryl® (Ethicon).

Statistics

Data were analysed by the Statistical Package for Social Science (SPSS) (SAS, 1995) using Wilcoxon signed rank test for non-parametric observations, correlation analysis (Spearman and Pearson) and logistic regression. For binary or ordinal response data, the logistic procedure was used.

Results

With experience, expressed by the number of surgical interventions, the duration of surgery decreased from 12 ± 2 min to 8 ± 1 min in the first 10 and last 10 animals respectively ($P = 0.0001$, Figure 1). Simultaneously the total adhesion score diminished as shown by the correlation between number of operations and adhesion score (Spearman: adhesion score 1, $P = 0.0001$; adhesion score 2, $P = 0.002$). This overall decrease in adhesion total scores with decreasing operation time was seen for the extent ($P = 0.0001$), tenacity ($P = 0.004$), type ($P = 0.002$) and inflammation ($P = 0.003$). This decrease of total adhesion scores was also seen at the individual lesions in the pouch of Douglas, i.e. the bipolar ($P = 0.05$), and the combination of both lesions ($P = 0.02$) and the uterine horn, i.e. the mechanical lesion ($P = 0.01$).

The duration of surgery also correlated with the total adhesion score (Spearman: adhesion score 1, $P = 0.002$;

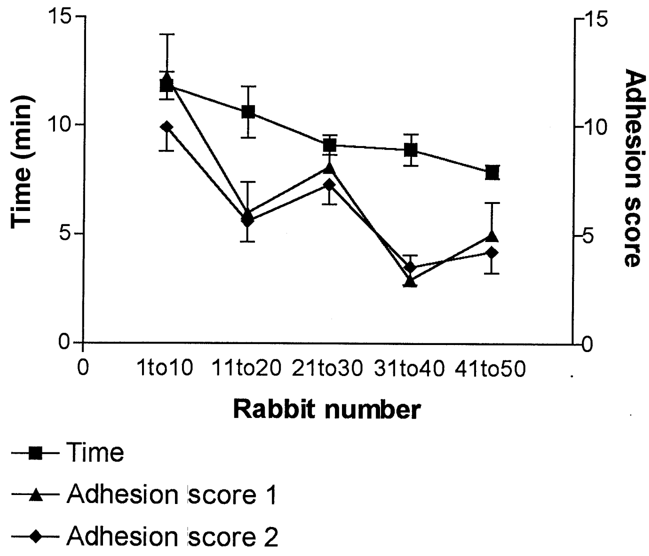

Figure 1. Effect of training on operating time and adhesion formation. Bars show SEM.

adhesion score 2, $P = 0.04$), with the extent ($P = 0.002$), the tenacity ($P = 0.0008$), inflammation ($P = 0.003$) but not with the total score of each individual lesion.

The amount of bleeding (no bleeding, $n = 13$; score 1, $n = 19$; score 2, $n = 12$; score 3, $n = 6$) did not correlate with experience or duration of surgery, nor with the total adhesion score, nor with extent, tenacity, type and inflammation, nor with the adhesion scores at the specific injuries.

The relative importance of experience, duration of surgery and bleeding were evaluated by logistic regression. Taken together, only experience expressed by the number of experiments performed was significantly correlated with adhesion scores (for adhesion scores 1 and 2, $P = 0.01$), suggesting that experience is more important than duration of surgery. According to adhesion score 2, at the uterine horn side wall the adhesion score was 2.2 ± 2.5 and 2.6 ± 2.7 following mechanical and bipolar lesion respectively (difference not significant).

At the uterine horn-side wall the adhesion score was 2.2 ± 2.5 and 2.6 ± 2.7 following a mechanical and a bipolar lesion respectively (difference not significant). In the pouch of Douglas, the total score was 1.2 ± 1.7, 2.3 ± 3.3 and 2.3 ± 2.5 following a mechanical, bipolar or a combination of both lesions respectively (difference not significant). Only the bipolar lesion at the uterine horn yielded slightly more adhesions than in the pouch of Douglas ($P = 0.05$).

The importance of inter-animal variability was suggested by the strong correlation between the adhesion scores in the pouch of Douglas, and around the uterine horn ($P < 0.001$). Moreover, by logistic regression the adhesion score in the pouch of Douglas could be predicted either by the combination of adhesion scores at the control sites ($P = 0.0004$), thus correcting for inter-animal variability the amount of bleeding ($P = 0.01$) and the duration of surgery ($P = 0.05$), or by a combination of the adhesion score at the control sites ($P = 0.002$) and the number of experiments ($P = 0.03$) and bleeding ($P = 0.08$).

Discussion

To the best of our knowledge, this is the first report demonstrating a relationship between postoperative adhesion formation and experience of the surgeon, expressed by the duration of surgery. This was not only observed for the total adhesion scores, but also for inflammation, extension, tenacity and type of adhesions. Experience and duration of surgery are so strongly interrelated, that both effects cannot be separated, i.e. it remains unclear whether experience, as an adhesiogenic factor, means more than duration of surgery only. The logistic regression showing that experience correlated systematically better with postoperative adhesions than duration of surgery suggests, however, that other factors such as gentle tissue handling and bleeding are probably important, since with experience the surgical procedure is less traumatic, more precise and as a consequence more haemostatic. Increased duration of surgery on the other hand could increase adhesions by CO_2 irritation through pH changes (Volz *et al.*, 1997), or by tissue dessication (Ryan *et al.*, 1973).

Inter-animal variability of postoperative adhesions has been observed in most studies as judged by the large standard deviations. In this study, following identical procedures some animals had almost no adhesions, whereas others developed a lot of adhesions. Although factors such as sub-clinical postoperative infections cannot be ruled out, the strong correlation between adhesions in the pouch of Douglas and at the uterine horn supports strongly this inter-animal variability. Since this inter-animal variation is an obvious difficulty in all adhesion studies the use of an 'internal standard', making each animal its own control, could be useful. This is illustrated by the fact that the effect of bleeding, a known adhesiogenic factor, did not reach statistical significance even after correction for duration of surgery or experience. Only after correction for inter-animal variability did the effect of bleeding upon adhesion score in the pouch of Douglas become significant.

The uterine horn yielded slightly more adhesions than the pouch of Douglas. These differences between the uterine horn and the pouch of Douglas in total adhesion scores could be caused by local steroid hormone effects through the proximity of the uterus to the ovary. The uterus is known to be very adhesiogenic (Adhesion Study Group, 1983; Diamond *et al.*, 1987).

For the evaluation of postoperative adhesions several scoring systems have been devised. All scoring systems take into account extent, tenacity, type and vascularization, but the points attributed to each factor remain arbitrary and none of these scoring systems have been validated. In 1988, the American Fertility Society proposed a scoring system for adnexal adhesions. In the same year Blauer and Collins proposed an adhesion scoring system which was related to the type of adhesions only. In 1995, Corson *et al.* presented a new scoring system for assessment of adhesions including type and extension. In 1996 Fielder *et al.* modified the scoring system of Boyers *et al.* (1988) in order to include extension, type, tenacity of the adhesions and inflammation. In this present study we used the latter scoring system and simultaneously a scoring system without inflammation and tenacity, but with specific scoring of the extragenital coverage (side wall, urinary tract and gastrointestinal tract). Although the overall conclusions of this investigation were similar for both scoring systems, some observations might be important. It is clear that, even after 7 days, adhesions can and should be scored for tenacity and inflammation, and that these parameters should be included in a scoring system. It is also obvious that the actual available scoring systems are not appropiated to evaluate factors such as CO_2 affecting the whole peritoneum.

Several animal models have been used to investigate adhesion formation, such as rats, rabbits and dogs. Rats have the advantage that inbred strains can be used, which limits inter-animal variability, but they are too small for extensive endoscopic procedures. Rabbits provide a well-established model for adhesion formation and are easily adapted to even complex endoscopic procedures with conventional equipment, such as nephrectomy and Nissen's operation (Luks *et al*., 1995).

In conclusion, in the rabbit laparoscopy model for the study of postoperative adhesions, there is a marked effect of experience and/or duration of surgery. These factors should be strictly standardized in all studies. Because of the strong inter-animal variability in rabbits, the use of a standard control lesion should be considered when local effects are investigated.

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