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Experimental Adhesion Prevention Studies: A Comparative Study in a Rabbit Model

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Competing Interest All authors declare no competing interests

ABSTRACT

Background: Adhesions formation after abdominal surgery is a problem that causes many complications in the patient's future life. In this study we compare the possible individual effects of Ringer's lactate, Adept® and Mesofol® and the combined effects of Adept® and Mesofol® in the prevention of post surgical adhesions formation in a standardised rabbit model.

Keywords: Post-operative adhesions; comparative study; prevention; rabbit model.

Materials and Methods: 90 rabbits were divided into 9 groups. The same experimental method was used in all rabbits to produce adhesions, consisting of cecal and sigmoid abrasion and excision of parietal peritoneum. The rabbits were operated after 2 weeks to assess the adhesions occurring. The results were analysed using the chi-square test and the Fisher exact test when needed.

Results: Comparison of adhesion stages demonstrated a significant difference between the control group and the Adept® groups ($p < 0.05$) in both branches. The adhesion grade of the combined treatment groups (G5 and G9) was not statistically significant ($p > 0.05$). In the Mesofol® group and the combined group, four and two rabbits, respectively, developed granulomas.

Conclusions: Adept® used individually reduced the adhesion grade. The Adept® and Mesofol® combination was less effective in reducing adhesion formation in comparison to the Adept® group by itself. On the other hand, in our experimental study, the usage of Mesofol®, alone or in combination, caused foreign body granulomas in 6 animals. Although the literature is very sufficient in comparison several materials for decreasing adhesion formation, we believe that our study provides more valuable evidence in this area. Due to the appearance of six cases of foreign body granulomas, detailed studies focused on this matter are needed in the future.

RESUMO

Introdução: A formação de aderências após a cirurgia abdominal é um problema que causa muitas complicações na vida futura do doente. Neste estudo, comparamos os possíveis efeitos individuais do Ringer lactato, Adept® e do Mesofol®, e os efeitos combinados do Adept® e do Mesofol® na prevenção da formação de aderências pós-cirúrgicas num modelo padronizado de coelhos.

Palavras-Chave: aderências pós-operatórias; estudo comparativo; prevenção; modelo animal em coelhos.

Materiais e Métodos: 90 coelhos foram divididos em nove grupos. O mesmo método experimental foi utilizado em todos os coelhos para favorecer a produção de aderências, realizando abrasão do cego e da sigmoideia e excisão do peritонеu parietal. Os coelhos foram operados após duas semanas para avaliar a ocorrência de aderências. Os resultados foram analisados por meio do teste do qui-quadrado e do teste exacto de Fisher, quando necessário.

Resultados: A comparação em estadios de aderência demonstrou uma diferença significativa entre o grupo controlo e os grupos Adept® ($p < 0,05$) em ambos os ramos. O grau de aderências dos grupos de tratamento combinado (G5 e G9) não foi estatisticamente significativo ($p < 0,05$). No grupo do Mesofol® e no grupo combinado, quatro e dois coelhos, respectivamente, desenvolveram granulomas.

Conclusões: Adept® utilizado individualmente reduziu o grau de aderências. O Adept® combinado com o Mesofol® foi menos eficaz na redução da formação de aderências em comparação com o grupo do Adept®, por si só. Por outro lado, o uso de Mesofol® isoladamente ou em conjunto podem aumentar o risco de granulomas. Estudos mais detalhados são necessários, e futuros estudos sobre a eficácia de um material para diminuir a formação de aderências deve incluir uma comparação de controle de diversos materiais num mesmo modelo



INTRODUCTION

Post surgical adhesion formation is a significant clinical problem causing pelvic pain, bowel obstruction and infertility (1,2,3). These adhesions are a frequently ensuing complication. Adhesions are frequent consequences of mesothelial repair at sites traumatised by surgical procedures where localised hypoxia suppressed fibrinolysis, enabling organization of fibrin deposits that persist between adjacent tissue surfaces (4-7).

Increased awareness of peritoneal adhesions has encouraged the use of surgical techniques such as laparoscopy, designed to minimise peritoneal trauma (8). Recent studies have demonstrated that adhesions continue to present a substantial burden following general surgery, despite careful attention to surgical techniques and the availability of adhesion-reduction strategies (9,10).

Physical barriers, including both mechanical and viscous solutions are used to try to prevent adhesions formation by limiting tissue opposition during the mesothelial repair.

The instillation of salt-containing solutions into the peritoneal cavity at the end of surgery in a volume of approximately (300-500ml) Ringer lactate solution has been the most popular option used in the past to prevent adhesions (2). However, hidroflotation with cristalloids has been shown not to reduce the formation of post-surgical adhesions in a meta-analysis of clinical trials (11).

Adept® is 4% Icodextrin, an alfa- 1,4 glucose polymer of high molecular weight which is rapidly metabolized to glucose by the alfa-amylase present in the systemic circulation, but is absorbed only slowly from the peritoneal cavity. The colourless, non-viscous, iso-osmolar 4% solution of Icodextrin, having an intra peritoneal residence time of at least 4 days (12), has the potential to reduce post-surgical adhesion formation by means of hidroflotation. Thus, general prophylactic use of adhesion prevention adjuvants requires clinical data from laparoscopic studies followed by second-look laparoscopy to assess efficacy.

Mesofol® is a resorbable sheet, described as use-

ful for the prevention of adhesions, fully absorbable and transparent, composed of lactide-caprolactone copolymers. So far, several positive experiences have been made with Mesofol® in the abdominal area during elective procedures. A clinical study for this indication is currently being carried out (13).

The purpose of the present study is to compare the efficacy of Ringer's lactate, Adept® and Mesofol® film in the prevention of post surgical adhesion formation in a standardized rabbit model.

MATERIALS AND METHODS

Animals

This study was conducted after approval by the Lisbon University School of Medicine's Ethics Committee and supported by the Surgical Department and the Experimental Surgery Unit of Santa Maria Hospital. 90 male New Zealand white rabbits weighing between 2,4-2,7 Kg were used, purchased and quarantined in the Experimental Unit vivaria of the Santa Maria University Hospital of Lisbon, for one week prior to its experimental use. The animals were kept in single cages under standard laboratory conditions on a 12 h dark cycle with food and water available *ad libitum*. The animals were randomly assigned for treatment in nine groups of ten rabbits per group.

Materials

The minimization of intra-abdominal adhesions was studied using Ringer Lactate, Adept® and Mesofol®. Ringer lactate solution, supplied by ROVI S.A, is an isotonic crystalloid solution that is widely used. The mechanism of action is not clear. It is supposed that the presence of a high volume of the solution in the abdominal cavity separates raw peritoneal surfaces and thus prevents adhesions. Adept®, when administered intraperitoneally as a 4% solution, functions as a colloid osmotic agent. This colloidal osmotic action



of icodextrin allows the retention of a reservoir of fluid within the peritoneal cavity for 3-4 days. It is believed to perform its function through a physical effect by providing a temporary separation of peritoneal surfaces by hydroflotation as the result of maintaining a fluid reservoir. This minimises tissue apposition during the critical period of fibrin formation and mesothelial regeneration following surgery, thereby providing a barrier to adhesion formation. Mesofol® Surgical Sheet, provided by BIOMET Europe through their Portuguese delegations is an implantable, resorbable, polymeric surgical sheet.

Experimental Adhesion Models

The animals of all experimental groups were anesthetized with a mixture of 55mg/kg Ketamine hydrochloride and 5 mg/Kg Ropum i.m. All surgical procedures were performed by the same researcher. Following preparation for sterile surgery, by hair removal and cleaning skin with 1% antiseptic povidone-iodine solution, a large midline laparotomy of 15 cm along the linea Alba was performed. The caecal and sigmoid regions were manipulated and traumatized by abrasions of the serosal surface with gauze until punctiform bleeding developed, and excision of a 5 cm x 3 cm area of parietal peritoneum of the left iliac region was made in groups 6 to 9. After the specific preparation of each group, the laparotomy was closed using 3-0 coated resorbable sutures. The animals of each group were sacrificed for study and (grading) of adhesions two weeks later.

Experimental Groups

BRANCH A (Visceral adhesion formation): Traumatic gut manipulation as described before and 2 weeks later were graded for adhesion formation.

Group 1 (Control experimental model A): Submitted to traumatic gut manipulation as described before.

Group 2 (Ringer's lactate): After gut manipulation 50ml of Ringer's lactate solution instilled into the abdominal cavity before closure.

Group 3 (Adept® group A): Traumatic gut manipulation and intra-abdominal instillation of 50 ml of 4% Icodextrin solution at the end of surgery.

Group 4 (Mesofol® group A): Traumatic gut manipulation and Mesofol® film application at the end of surgery.

Group 5 (Adept® and Mesofol® combination group A): Traumatic gut manipulation and instillation of 50 ml of 4% Icodextrin and Mesofol® film application.

BRANCH B (Visceral adhesion formation + Sidelwall adhesion formation): Traumatic gut manipulation plus excision of 5cm x 3cm area of parietal peritoneum of the left iliac region, 2 weeks later were graded for adhesion formation.

Group 6 (Control experimental model B): Submitted to traumatic gut manipulation, parietal peritoneal excision as described before.

Group 7 (Adept® group B): Traumatic gut manipulation, parietal peritoneal excision and intra-abdominal instillation of 4% Icodextrin solution.

Group 8 (Mesofol® group B): Traumatic gut manipulation, parietal peritoneal excision and Mesofol® film application.

Group 9 (Adept® and Mesofol® combination group B): Traumatic gut manipulation, parietal peritoneal excision and instillation of 50 ml of 4% Icodextrin and Mesofol® film application.

Assessment of Adhesion

2 weeks after surgery, the abdominal cavity was opened for complete exploration and adhesion formation evaluated and scored according to the defined scale by two independent observers who were blinded to the treatment of the animal. At the end animals were sacrificed. Evaluation of adhesions was graded from 0 (Absent) to 3 (Severe) according to the classification. (Table 1)





Figure 1. Caecal and sigmoid abrasions after traumatized serosa with punctiform bleeding

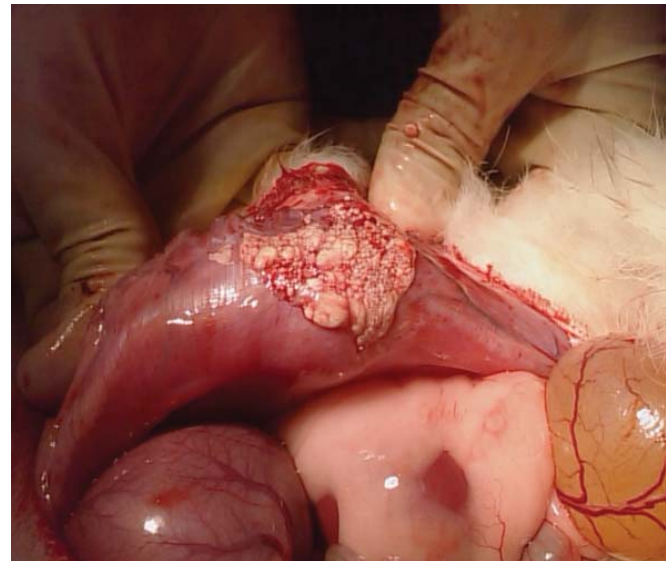


Figure 2. Local foreign body granuloma on the wall

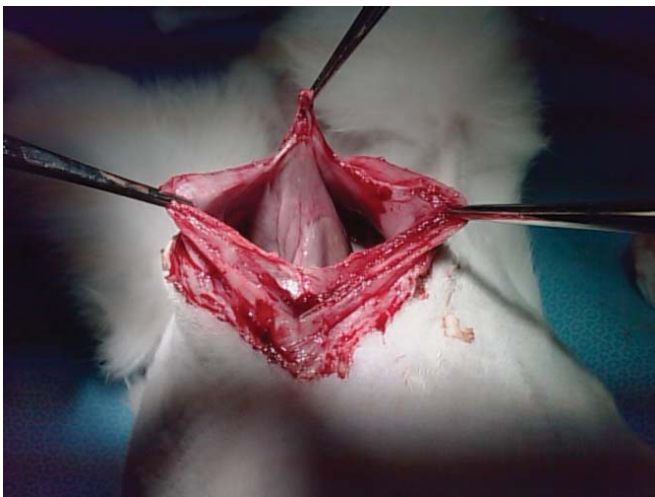


Figure 3. Side wall adhesion

Statistical Analysis

A statistical analysis was performed using the Statistical Package for Social Science (SPSS) for Windows version 17.0 (SPSS Inc., Chicago, IL). A statistical analysis regarding incidence and score of adhesions, infection and mortality rates was done, eventually, using a chi-square test and in certain cases a Fisher

exact test was carried out. A value of $p < 0.05$ was defined as significant.

RESULTS

Throughout the investigation, no animal died during or after surgery. Adhesion rates and grades of the groups are presented in Table 1. A comparison of adhesion grades demonstrated a significant difference between the control group and the Adept® groups (G3 and G7) ($p < 0.05$) in both branches (Table 3). However, the rest of the groups did not show any significant differences compared to Control Group (G1) ($p > 0.05$). On the other hand, the results of group 5 and group 9 (Adept® and Mesofol® combination group), was not statistically significant ($p < 0.05$) in both branches. Adhesion rates and grades of the groups are presented in Table 2.

In the control groups (G1 and G6) there were no adhesions in grade 0. Furthermore, most adhesions in the control groups were moderated or dense (90%). Group 3 and 7 showed the lowest incidence of post-surgical adhesions (50%) when compared to other treatment and control groups ($p < 0.05$). In groups



Table 1 – Adhesion grading

Grade	Description of grade
0	No adhesions
1	Mild, easily dissectable adhesions
2	Moderate adhesions; non-dissectable, does not tear the organ
3	Dense adhesions; non-dissectable, tear the organ when removed

Table 2 – Postoperative adhesions rates, grades and side effects for groups

Groups	Adhesions					% adhesions formation
	<i>n</i> Total	No adhesions	Mild	Moderate	Dense	
Group 1	10	0	2	4	4	100%
Group 2	10	1	3	1	5	90%
Group 3	10	5	1	1	3	50%
Group 4	10	3	0	3	4	70%
Group 5	10	3	1	2	4	70%
Group 6	10	0	0	4	6	100%
Group 7	10	5	1	0	4	50%
Group 8	10	2	1	2	5	80%
Group 9	10	3	3	1	3	70%
Total	90	22	12	18	38	



4 (Mesofol® alone) and 5 (Mesofol® combined with Adept®), four and two rabbits, respectively, developed local foreign body granulomas on the wall (Figure 2).

DISCUSSION

Postsurgical adhesion formation is a significant clinical problem for every surgical specialty. The formation of peritoneal adhesions after laparotomy continues to represent a significant clinical and economical burden. Great efforts continue to be made to elucidate the possible causes and thus, to allow applying preventive measures. However, no complete success has been achieved yet.

Evaluation of the causes and means of prevention of adhesion formation has been the major goal of many investigations. Research in adhesion prevention has focused strongly on barrier films, fibrinolytic agents and phospholipids (14-19).

There are several reports on the use of Icodextrin and Ringer's for the prevention of peritoneal adhesions (8, 20,21). We were able to significantly reduce the incidence and degree of adhesions in rabbits using Icodextrin 4% solution instillation at the end of surgery.

The incidence of adhesions was reduced by 50 percent with Adept® alone. By contrast, when associated with Mesofol® the reduction of adhesions was 30 percent in both branches. It is possible that the foreign body granuloma plays a role in this results.

After peritoneal trauma there is an activation of the fibrinolytic activity due to surgery. This activates inflammatory reaction, which leads to the formation of exudates rich in fibrin. When there is an overproduction of fibrin, the fibrinolytic capacity of the peritoneum is overtaken resulting in deposits of fibrin which reflects in adhesions capacity.

With the barrier technique, surgically traumatized surfaces are kept covered during mesothelial regeneration, thus preventing adherence of adjacent structures and reducing adhesion formation. However, consid-

ring the functional properties of Icodextrin 4%, we believe that it acts, probably as a barrier, preventing the development of adhesion formation.

Table 3 – Statistical comparison of the groups according to the adhesion grade

Versus	P value
G1 Vs G2	>0,05
G1 Vs G3	0,033
G1 Vs G4	>0,05
G1 Vs G5	>0,05
G1 Vs G5	>0,05
G6 Vs G7	0,033
G6 Vs G8	>0,05
G6 Vs G9	>0,05

A value of $p < 0.05$ was taken as significant

Preliminary clinical studies have demonstrated that Icodextrin 4% solution is an effective treatment for the reducing adhesion formation following surgery (21,22).

ARIEL (Adept® Registry for Clinical Evaluation) is a Europe-wide registry that was established to collate feedback on the clinical experiences of general and gynaecological surgeons regarding their use of Icodextrin 4% solution during routine laparotomy and laparoscopy (20). The registry aims to provide an information source for surgical centres on the optimal usage and safety of Icodextrin 4% solution in routine surgery.



In the present study, Adept® significantly reduced adhesion formation ($p < 0.05$). There was no adhesion formation of Grades 3 or 4 in the Adept® group (Group 2). In fact, most of the adhesions in Group 2 developed in uncovered areas in the abdomen. This fact underlines the necessity of using liquid anti-adhesive agents to cover all potential peritoneal lesions.

Because Adept® is the only material that has been studied extensively in well-designed randomized controlled clinical trials,(20,23) we preferred to use Adept® for comparison with the other agents used in this study.

CONCLUSION

In conclusion, Adept® used in isolation reduced the adhesion grade; the combination of Adept and Mesofol® was less effective in reducing adhesion formation. More detailed studies are needed on this topic, and future studies on the efficacy of a material in decreasing adhesion formation should include a comparison of several control materials.

Based on these findings, we can conclude that only the Icodextrin 4% reduced significantly peritoneal adhesion development rate and adhesion grades.

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