

Hyaluronic acid and adhesions on polypropylene endoperitoneal mesh

An experimental study



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INTRODUCTION: *The aim of this study was to verify the effectiveness of hyaluronic acid in preventing adhesion formation after endoperitoneal surgery in which prosthetic polypropylene mesh is placed directly on the viscera.*

Methods: *Forty albino rats were included in this study and the animals were randomized to the following 4 groups each with 10 rats: polypropylene prosthesis (PP), PP+hyaluronic acid (HA), Hertra prosthesis (HP), HP+HA. A large defect was created in the anterior abdominal wall of each rat and repaired in different ways. In the first group a polypropylene mesh was placed intraperitoneally, while, in the second group the peritoneal surface of the mesh was impregnated with HA. In the third group a Hertra 0 polypropylene rigid mesh was placed intraperitoneally and, in the fourth group, the peritoneal surface of the Hertra 0 mesh was impregnated with HA. Clinical controls on the animals were carried out at 1 month. Each group was divided into two subgroups in which the controls and the prosthetic explantation were randomly carried out at 3 and 6 months. Explanted prostheses were subjected to histological and immunohistochemical analysis, and examined for shrinkage. An assessment of adhesion formation was performed, evaluating the quantity and tenacity of the adhesions.*

RESULTS: *We demonstrated higher levels of adhesions in rats with PP than in those with HP and lower levels in rats with a protective layer of hyaluronic acid. The amount of fibronectin in the periprosthetic fibrotic tissue and the histological score confirmed the previous data.*

CONCLUSIONS: *Hertra 0 mesh with HA provided the best results in terms of physical stability and resistance to adhesion formation.*

KEY WORDS: Hyaluronic acid, Peritoneal adhesions, Polypropylene, Shrinkage, Ventral hernia repair

Introduction

Surgery of the abdominal wall is in constant evolution, because of important new technical changes introduced in the last thirty years that have radically altered the tra-

ditional approach. The introduction of polypropylene prostheses has modified surgical technique, leading to better results ¹. Today the Rives technique is the gold standard for prosthetic repair of incisional and epigastric hernias, because it involves tension-free repair and compared to previous techniques drastically reduces the incidence of relapses ²⁻⁵. The ideal mesh for open surgery using a sutureless technique is a rigid and shape-memory device like Hertra 0 mesh. When it was not possible to close the margin of posterior aponeurosis of rectus abdominis muscle and parietal peritoneum, intraperitoneal prosthetic repair techniques that could require a simple laparoscopic execution were used ⁶⁻¹⁰. However, the intraperitoneal insertion of polypropylene mesh, causes ventral adhesions, ¹¹⁻¹³ that can be defined as abnor-

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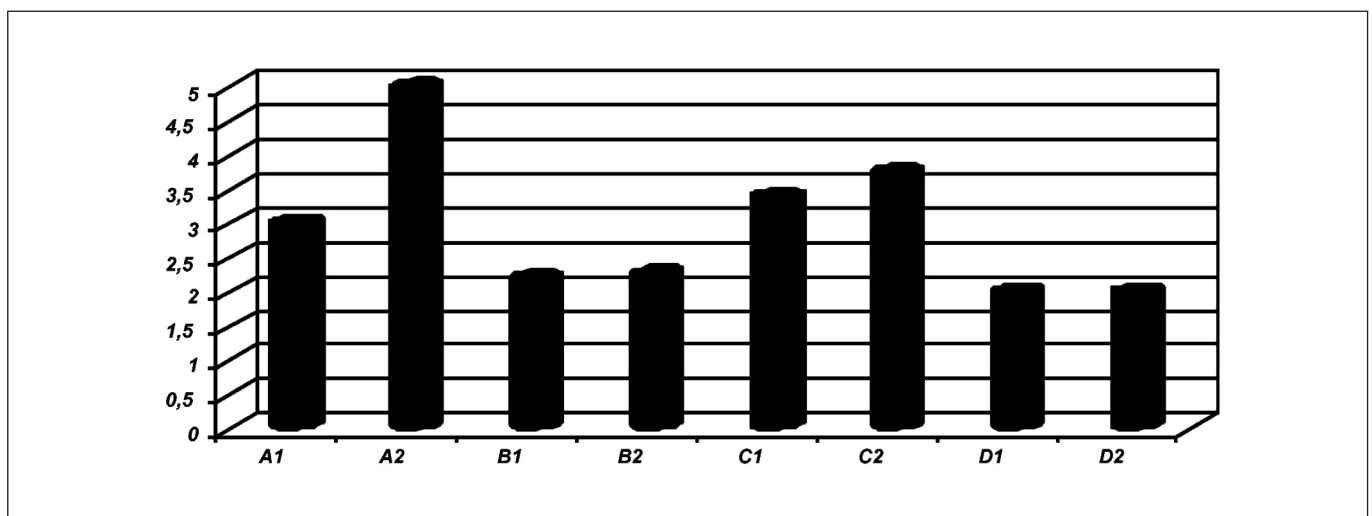
mal fibrous bands between organs or tissues or both in the abdominal cavity that are normally separated. It has been estimated that 93% to 100% of patients who undergo transperitoneal surgery will develop postoperative adhesions^{14,15}. The extent of adhesion formation varies from one patient to another and depends most on the type and magnitude of the surgery performed, as well as any postoperative complications. This condition is not always symptomatic, but in some cases causes pain, small bowel obstruction and fistula. Several strategies have been proposed to reduce the incidence of postoperative adhesions, like the development of new multilayer prostheses which do not easily adhere to the viscera. These devices have properties that ensure perfect adhesion to the peritoneum on one side while minimizing adhesion to the viscera on the other side. However use of these composite devices, involves high costs and a general risk of adhesions.¹⁴ So over the years many agents have been proposed to prevent surgical adhesions and in recent years, studies have focused on the use of biological materials such as hyaluronic acid (HA) that is present in cartilage and skin. Hyaluronic acid, a naturally derived polysaccharide, has demonstrated excellent biocompatibility and low cytotoxicity in various studies¹⁶. Limitations of HA include its rapid clearance from the abdominal cavity (about 12 hours) and the lack of large-scale clinical trials. Recently Hyalobarrier Gel® (Nordic Group) a highly viscous gel containing hyaluronic acid derivatives, has been developed which has been shown to reduce the incidence and severity of postoperative adhesions. The aim of our study was to verify the effectiveness of hyaluronic acid used for adhe-

sion prevention after prosthetic endoperitoneal surgery with polypropylene mesh prostheses placed directly on the rats' viscera.

Materials and methods

The experimental study was approved by the Institute of Animal Care of the Faculty of Medicine at the Second University of Naples, and conducted in accordance with the National Institute of Health guidelines for use of experimental animals. The subjects were 40 albino rats, New Zealand stock, with an average weight of 413 g (standard deviation: 77.2); housed in suitable environmental conditions (12 hours daylight, 22°C temperature) with food and water ad libitum. The animals were randomly divided into 4 groups, with 10 rats each: the polypropylene prosthesis (PP) group (Group A), the PP+hyaluronic acid (HA) group (Group B), the Hertra prosthesis (HP) group (Group C), and the HP+HA group (Group D). A large defect was created in the anterior abdominal wall of each rat. Intramuscular ketamine hydrochloride (0.02 mg/ kg⁻¹) and atropine (0.01 mg/ kg⁻¹) were used to induce anesthesia, then intramuscular fentanyl (0.02 mg/ kg⁻¹) was administered. The animals were considered to be anesthetized when unconscious and without voluntary movements. Antibiotic prophylaxis consisted of intramuscular ceftriaxone (0.02 mg/kg⁻¹). All animals were operated on by the same surgical team. The abdomen was shaved and cleaned with 2 % iodine; then it was opened through a 1 cm midline incision, and a sterile piece of

TABLE I - Clinical score (average of the population, determined by three operators)



Legend: A1: polypropylene at 3 months, A2: polypropylene at 6 months, B1: polypropylene and hyalobarrier at 3 months, B2: polypropylene and hyalobarrier at 6 months, C1: Hertra at 3 months, C2: Hertra at 6 months, D1: Hertra and hyalobarrier at 3 months, D2: Hertra and hyalobarrier at 6 months.

2 cm mesh was placed in a sublay position (intraperitoneally) and fixed with four sutures. The abdominal wall and the skin were closed with a running suture. In Group A, a PP, previously marked by 4 hemostatic radiopaque clips placed in the cardinal points, was positioned intraperitoneally, and fixed to the abdominal wall with 4 stitches of 3-0 polypropylene. In Group B the same procedure was performed, but the peritoneal side of the mesh was impregnated with HA gel (Hyalobarrier Gel ®). In Group C the same procedure was performed, this time using an "Hertra 0" prosthesis (Herniamesh ® Italy), that is rigid, with shape-memory features, because it has a more dense interlacing of polypropylene mesh. In Group D, HP impregnated with HA were used.

All animals were subjected to a radiological control immediately after surgery and at 1 month to check if the prosthesis was correctly positioned, and to measure, empirically, the degree of "shrinkage". Each group was divided into 2 subgroups, to be monitored at 3 and 6 months. After a period of observation the animals were anesthetised and sacrificed.

Independent observers, who were blinded to the study group, assessed adhesion coverage of the mesh surface using a semi-quantitative scoring system according to the modified Diamond scale (0 = no adhesion, 5 = maximum degree of adhesion) ¹⁷⁻¹⁸.

The explanted prostheses were then studied in the Department of Pathology of the Second University of Naples, where a third observer, who didn't know to which group the analyzed mesh belonged, performed a semi-quantitative study of fibronectin with optical and electron microscopy, taking an average of 8 samples per prosthesis, examining them at 60x magnification and giving a histological score (Table I) for the quantity of new tissue to classify the degree of periprosthetic fibrosis. The histological score, according to the modified Diamond scale, goes from 1 to 5 (1= almost no new tissue, 5 = mesh unrecognizable within fibrotic tissue). Statistical analysis was carried out using the EpiInfo 3.5 database, and the Mann-Whitney U test. $P < 0,050$ was considered significant.

Results

During the study 3 animals died, for reasons unrelated to the group they were in (1 death in Group A1 group, 1 death in Group A2, 1 death in Group D2).

The average clinical score (according to the modified Diamond scale) for each group is given in Table II. The average histological score for each group is given in Table 3. No modification of the position of the prostheses was observed on radiological examination. Modifications of mesh size were observed; there was, on average a 10% increase in Groups A and B but only insignificant modifications of mesh size in groups C and D.

TABLE II - Average clinical score

Group A1	3/5 (SD 0,82)
Group A2	5/5 (SD 0,58)
Group B1	2,2/5 (SD 0,84)
Group B2	2,25/5 (SD 0,96)
Group C1	3,4/5 (SD 0,55)
Group C2	3,75/5 (SD 1,5)
Group D1	2/5 (SD 1)
Group D2	2/5 (SD 0,82)

(SD: Standard Deviation).

Discussion

There is great support in the literature for cutting the costs of intraperitoneal techniques, shown particularly in the biotesting of materials that have the same peculiarities as the multilayer meshes to prevent the adhesions without excessive expense. Many authors, in fact, have tested on experimental models the effects of various types of prosthesis made with aid of bioabsorbable materials, on reducing adhesion formation, but without any follow-up after 2 months¹⁹⁻²³.

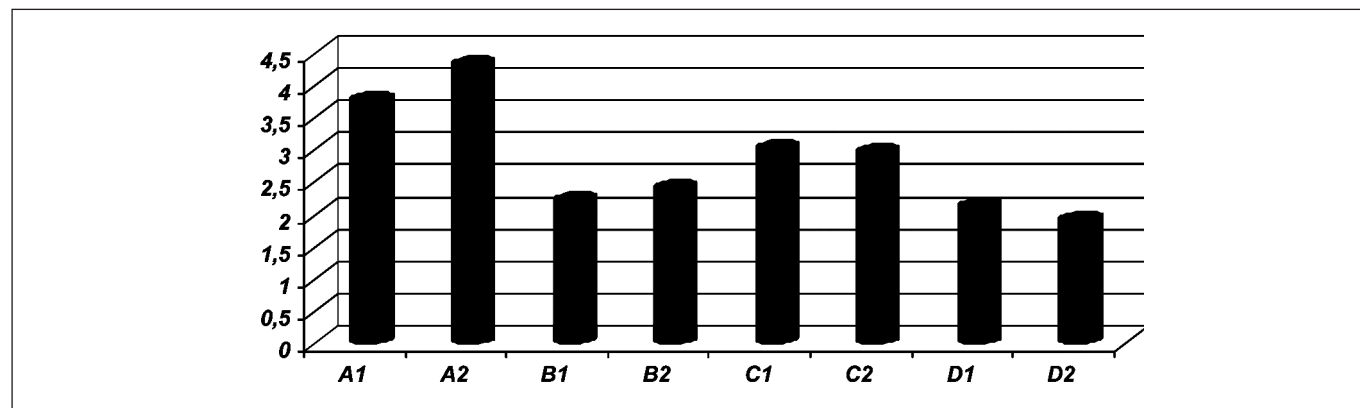
A recent experimental study on the effects of fibrin glue applied to the peritoneal surface of polypropylene mesh, found that the quantity and tenacity of adhesions was reduced, but had a short follow-up lasting only about 5 weeks²⁴.

Our Study design wants, therefore, to assure a longer lasting follow-up of the operated animals (3 and 6 months), to allow the full consolidation of the newly formed adhesions. Analysis of the clinical scores, and comparison of the fibronectin study results with the histological scores, shows that the observations were similar. In a single-blind study, this is an additional indication of the accuracy of the results. Comparison adhesion formation at 3 and 6 months, revealed a notable reduction of all adhesion indices examined, when HA had been applied to the prostheses, in contrast to results of studies in the literature which used different clinical and histological means for scoring.²⁵⁻³² Some authors obtained similar data, but only until 2 months after prosthesis implantation.

Apart from Group A (soft prosthesis, uncoated), there were no significant differences in the averages of the score between the three-month and six-month implant. Independently of the presence of HA, there were differences in the adhesiveness of the polypropylene prosthesis, used as a control, and new Hertra 0 prosthesis, tested in this study.

In our experience, Hertra 0 meshes represent the best compromise between rigidity and adhesiveness³³. The Hertra 0 mesh we used was rigid and, because of its rigidity and shape-memory features it could be easily positioned without fixation, and was really "tension-free",

TABLE III - Histological score (average of the population, 8 samples per specimen, magnification 400x)



Legend: A1: polypropylene at 3 months, A2: polypropylene at 6 months, B1: polypropylene and hyalobarrier at 3 months, B2: polypropylene and hyalobarrier at 6 months, C1: Hertra at 3 months, C2: Hertra to 6 months, D1: Hertra and hyalobarrier at 3 months, D2: Hertra and hyalobarrier at 6 months.

in contrast to standard polypropylene mesh. This greater resistance to flexion was due to narrower mesh, which also reduced porosity and reduced the probability of adhesion development. This mesh design explains the difference in adhesion scores found in our study.

Radiological measurement of the size of the prosthesis, even if it was a rather empiric method of evaluating the degree of shrinkage, made possible further assessment of the physical characteristics of the Hertra 0 prosthesis. Two months after implantation there were no essential variations in the size of the prosthesis³⁴.

This data, given the special flexibility afforded by a design with narrow mesh, is in agreement with the data of Coda and colleagues³⁵, who, in a clinical experimental study, published in 2000, reported variations in the size of different types of prostheses between -40% and +58.5%, and recorded minor variations for the Hertra 2 prosthesis (-6.1% / +6.7%), due to the fact that it has a denser texture than the other polypropylene meshes examined.

The variations in the size of the different types of prosthesis examined, which reached very significant values, could be the reason there is a high percentage of relapses. Shrinkage of Dual Mesh® (expanded polytetrafluoroethylene) in a recent study³⁶ was remarkably lower, with a mean of 7.5%.

The reduced tendency of the Hertra 0 prosthesis to develop adhesions, was enhanced by the application of HA, and a smaller clinical and histological score was obtained when both products were used^{37,38}.

Conclusions

The rigid Hertra 0 prosthesis has given better results, in terms of physical stability of the implant, and has shown

a greater resistance to the formation of adhesions in comparison with the polypropylene meshes normally used. Use of the Hertra 0 prosthesis with HA resulted in lower adhesion scores, with data at 6 months that proved to be stable. We believe that a more extensive study should be conducted to test the validity of our results. Repair of large abdominal defects with Hertra mesh and HA would be extremely advantageous, because the costs of the prosthesis and the protective membrane are significantly lower than the costs of the more complex solutions in use today.

Riassunto

INTRODUZIONE: Lo studio ha voluto verificare l'impiego dell'acido ialuronico nella prevenzione delle aderenze post-operatorie dopo posizionamento di protesi di polipropilene endoperitoneali.

METODI: Sono stati utilizzati 40 ratti albini sottoposti alla creazione di un ampio difetto della parete muscolare anteriore e del peritoneo divisi in 4 gruppi come modalità di ricostruzione: nei primi 10 ratti si è posizionata una rete di polipropilene intraperitoneale, nel secondo gruppo la rete preperitoneale è stata impregnata lungo il versante peritoneale con acido ialuronico. Nel terzo gruppo di ratti è stata posizionata una rete Hertra 0 di polipropilene rigida mentre nel quarto gruppo la rete Hertra veniva impregnata nel versante peritoneale con acido ialuronico. I ratti sono stati controllati a 1 mese ed ogni gruppo è stato diviso in 2 sottogruppi a 3 e 6 mesi in cui si procedeva ad espanto della protesi per gli esami istologici ed immunoistochimici. È stata quantificata quindi l'entità della formazione di aderenze post-operatorie.

RISULTATI: Abbiamo rilevato maggiore entità di aderenze

nei ratti con rete di polipropilene rispetto alla rete Hertra e bassa entità di aderenze nei ratti in cui era stata posizionato l'acido ialuronico.

CONCLUSIONI: La rete Hertra 0 con acido ialuronico ha fornito migliori risultati in termini di stabilità e resistenza alla formazione di aderenze.

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