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Effect of Sirolimus on Intra-Abdominal adhesion development in a rat model

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ABSTRACT

Objective: Postoperative intraabdominal adhesions still cause significant morbidity in surgical patients. This study aims to evaluate the effects of an immunosuppressor known as Sirolimus and an antiadhesive membrane which is formed with sodium hyaluronate carboxymethylcellulose-based bioresorbable membrane (Seprafilm™) to the intraabdominal adhesion formation in a rat model.

Materials and Methods: This experimental study was performed at an experimental research center, Yeditepe University Faculty of Medicine, Istanbul. Spraque-Dawley Rats, at a weight of about 250±20 gr, were used. Group 1 (n=8): Abdomen was closed after applying cecal abrasion (control group), group 2 (n=8): 10 x 30 mm Seprafilm™ was applied under the abdominal wall after cecal abrasion (Seprafilm™ group). Group 3 (n =8): Sirolimus (0,5 mg/kg) was applied (Sirolimus group). Adhesions quantitatively evaluated by a blinded assessor according to the classification of Nair and his colleagues.

Results: Statistically significant difference in terms of adhesion severity scores according to the Nair classification was found between the Sirolimus and the control group (p=0,03). Whereas, no statistically significant difference was found between the Seprafilm™ and the control group (p=0,17). Similarly, no statistically significant difference was found between Seprafilm™ and sirolimus group (p=0,64).

Conclusion: Although there was no statistically significant difference between intraperitoneal application of Sirolimus and Seprafilm™ group (p = 0.57), a statistically significant difference was found when each group compared with the control group (p=0,03). Combined anti-adhesive effect of Sirolimus and Seprafilm™ can be evaluated in future studies.

Keywords: Abdominal Adhesion, Experimental study, Sirolimus, Seprafilm

INTRODUCTION

Together with improved anesthesia and surgical techniques, the frequency of abdominal operations have kept on increasing nowadays, and due to this reason increase in postoperative intraabdominal adhesions have also been observed. The main surgical problems caused by adhesions are intestinal and enterocutaneous fistula formations, difficulty in relaparotomy procedures, and infertility in women. An agent having ideal characteristics that completely prevents adhesions could not be discovered yet, but this subject is one of the current research subjects. Following abdominal operations, the frequency of intraabdominal adhesions has been reported in varying rates between 67% to 93% (1). In a study, it has been reported that 5,5% of all of the hospital applications that have been made adhesion (2). The most severe complication of intraabdominal adhesions are intestinal obstructions. This situation is observed in 1-3% of the patients that apply to the general surgical clinics (3, 4). In addition to the medical problems that are caused by adhesion, its cost also leads to severe problems. More than 300,000 procedures are carried out every year for adhesiolysis in the United States of America (USA), and 1.3billion $ are spent annually for direct patient care related to said procedures. In England, the annual medical expenses caused due to adhesive small intestine obstructions add up to 12 million £ (5, 6).

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Several studies have been carried out regarding this first step of adhesion development until today, and although favourable results have been obtained, many results also had unwanted effects. As fibroblasts form the dominant cell type in the medium, in the latent period following the early inflammation phase, the usage of agents that may prevent the migration or activity of these cells has broadened up the horizon in preventing intraabdominal adhesions (**6**).

In this study, we aim to evaluate the effects of Sirolimus and sodium hyaluronate carboxymethylcellulose-based bioresorbable membrane (Seprafilm™) to the intraabdominal adhesion formation in a rat model.

MATERIAL and METHODS

This empirical study was accepted at the meeting dated 02.05.2011 of Yeditepe University Laboratory Animals Ethic's Committee and received confirmation with the decision number 187 of the Laboratory Animals Ethic's Committee. This empirical study has been carried out in the Emrpical Research Center of the Medical Faculty at Yeditepe University (YÜDETAM). Spraque-Dawley Rats with weights between 250±20 gr have been used as laboratory animals, which were bred in YÜDETAM and were fed with standard rat feed and water ad libitum. All of the animals were not fed food or water for 12 hours before a surgical operation. Three groups formed Group 1 (n=8): Control group, Group 2(n=8): Group treated with SeprafilmTM following cecal abrasion, Group 3(n=8): Group treated with Sirolimus following Cecal abrasion.

Operation Technique

The operations were carried out under semi-sterile conditions. Anaesthesia was established by intramuscu8lar injection of 50 mg/kg Ketamine hydrochloride (Ketalar, Pfizer İlaçları Lmt. Company, Istanbul, Turkey) and 4 mg/kg xylazine (Rompun, Bayer Türk Kimya San. Ltd. Company Istanbul, Turkey). During the trial, spontaneous respiration of the rats was enabled. A table lamp was used in order to maintain the body temperatures of the rats at 37˚C. All of the animals were shaved and cleaned and wiped with povidone-iodine. Laparotomy was carried out with a 25mm midline incision. The subjects were randomized to 3 different groups according to a random number table after abrasion was established in the first operation.

The aim of this operation was to form adhesion at a broad spectrum. A 20mm midline incision was made to the abdomen (**Figure 1**). The Cecum was found and taken out from the abdomen. The Cecum and the small intestines were carefully placed on wet gas, and some areas were abraded with a toothbrush. While this procedure was being carried out, only serosal injuries were created. The cecum front wall and different sections of the ileum intestine segment were abraded in all subjects using this method. After the Cecum and small intestine segments were placed in their first places, the abdomen wall was sealed with 4/0 absorbable suture (Vicryl) and 3/0 silk with double layers. At this stage, the subjects were randomized into the three different groups mentioned above.

1- In group 1 (n= 8), cecal abrasion was applied, and the abdomen was sealed (control group).

2- In group 2 (n=8), Following cecal abrasion, SeprafilmTM was applied (SeprafilmTM group) comprising 10x30mm hyaluronidase and carboxymethyl cellulose beneath the abdomen wall.

3- In group 3 (n=8), following cecal abrasion Sirolimus (0,5 mg/kg) was applied (Sirolimus group).

None of the rats that were included in the three groups monitored during the postoperative phase were lost due to anaesthesia. All of the rats were sacrificed with a high dose of ether in compliance with the Helsinki agreement on the 14th day. Furthermore, following this, U incision was made on their abdomens, and the abdomen walls were retracted downwards in order to provide a maximum view. Afterward, through the classification defined by Nair et al., the adhesions were quantitatively evaluated (**Table 1**) (**7**). **Figure 2** and **3** show macroscopic grade of adhesions. The evaluation was carried out as double-blind in compliance with the classification provided to a blind examiner.

Histopathological Evaluation

Pathological pieces were fixed in cups containing 10% buffered formol. The pieces that were monitored with classic laboratory methods were embedded into paraffin blocks. They were stained with hematoxylin-eosin and were examined with light microscopy (**Figure 4 and 5**). The pathologist that carried out the examination did not know which group the pieces were taken from. Following the histopathologic evaluation, the pieces were subjected to microscopic classification defined by Zühlke (**8,9**) (**Table–2**).

Statistical Evaluation

Statistical Packages for Social Science (SPSS 15,0 for Windows Evaluation) program was used in order to evaluate data and to carry out comparisons between groups. A chi-square test was carried out for categorical data analysis. Values less than 0,05 were accepted to be statistically significant p values.

RESULTS

When the group applied with Sirolimus was compared with the Control group, a statistically significant difference was found (p=0,03) in terms of adhesion severity scores, according to Nair (**Table 3**) classification. However, despite this, a statistically significant difference was not observed (p=0,17) between the control group and the group treated with SeprafilmTM. Similarly, a statistically significant difference was not observed between the group treated with SeprafilmTM and the group treated with Sirolimus (p=0,64).

While statistically significant difference (p=0,03) was found when the group treated with Sirolimus was compared in terms of microscopic adhesion grading of the control group similar to macroscopic grading during the histopathologic evaluation, and when compared with the group treated with SeprafilmTM a statistically significant difference could not be found (p=0,09). At the same time, a statistically significant difference (p=0,57) could not be found between the group treated with SeprafilmTM and the group treated with Sirolimus.

The values obtained by using Zuhlke classification during the histopathologic evaluation were shown in **Table 4**.

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Figure 1: Abdominal Incision

Table 1: 'Nair' macroscopic adhesion classification

Grade 0: No ahdesion
Grade 1: A single adhesive band between the organs or between the organ and the abdomen wall
Grade 2: Two adhesive bands between the organs or between the organ and the abdomen wall
Grade 3: More than two adhesive bands between the organs or between the organ and the abdomen wall or intestinal adhesions without adhesion to the abdomen wall
Grade 4: The viscera being directly adhered to the abdomen wall

Figure 2: Macroscopic grade 1 adhesion

Figure 3: Macroscopic grade 4 adhesion

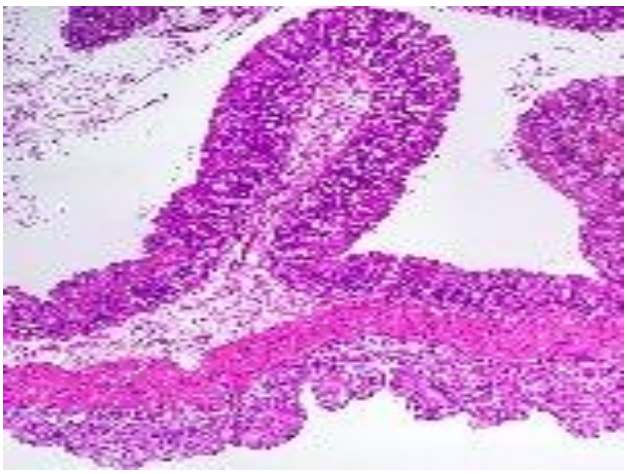

Figure 4: Microscopic image of inflammatory cell infiltration (Haematoxylin-eosin staining x100)

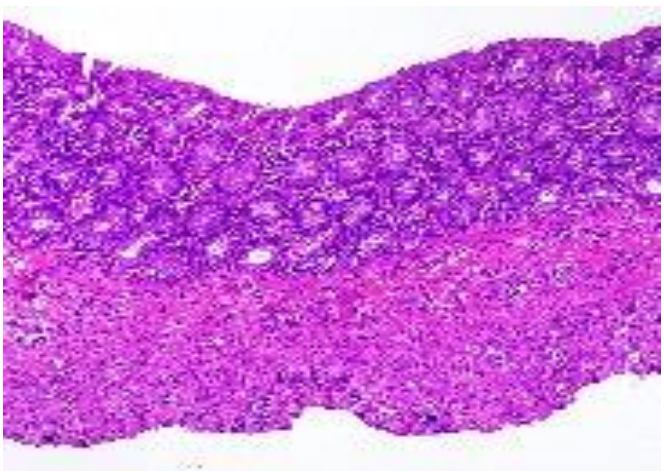

Figure 5: Microscopic view of Fibrosis (Haematoxylin-eosin staining, x100)

Table 2: 'Zühlke' microscopic adhesion classification (grading)

Grade 1: Weak connective tissue, abundant cell, old and new fibrin, thin reticular fibrils
Grade 2: Connective tissue with cells and capillary vessels, rare collagen fibers
Grade 3: Thicker connective tissue, rare cells, and more vessels, rare elastic, and straight muscle fibers
Grade 4: Old thick granulation tissue, poor in terms of cells, difficult separation of serosal layers.

Table 3: Adhesion severity scores according to Nair classification

Adhesion Score	Group 1 (n=8)	Group 2 (n=8)	Group 3 (n=8)
0	0	2	3
1	1	3	4
2	2	2	1
3	2	1	0
4	3	0	0

Table 4: Microscopic adhesion grading according to groups

Subject	Control	Seprafilm™	Sirolimus
1	3	2	1
2	4	3	1
3	3	1	2
4	2	2	2
5	2	2	1
6	2	1	2
7	4	1	2
8	4	2	2

DISCUSSION

Postoperative intraabdominal adhesion is one of the basic problems that cause severe morbidities such as pelvic pains, infertility, intestinal blockage, and ureter obstructions (**10, 11**). Besides the additional morbidity, it poses for patients, it is also a financial burden for countries' economies. A method that prevents abdominal adhesion prevents repeating operations, and the morbidity and financial burden said operations bring about (**8**). There are some studies, in order to prevent adhesions that have been carried out (**5,12**). Although laparoscopic techniques and minimally invasive surgery have been adopted in order to reduce the trauma that may occur during a surgical intervention, surgical technique on its own is not sufficient to reduce postoperative adhesions and complications related to adhesions (**6**). As the improvement of surgical procedure had its limitations, physical barriers, and the usage of pharmacologic agents in order to prevent adhesion formation have come into prominence. Large and small peritoneal defects heal at the same time, and mesothelial healing is completed within seven days (**9**). In contemporary approaches in order to prevent adhesions, peritoneal damages must be reduced during operation (minimal, invasive, surgical), the inflammatory response must be reduced, coagulation inhibition must be provided, fibrinolysis must be stimulated, and adhesion surfaces must be separated (**13**). The treatment strategies in the future in order to prevent adhesion; must aim to control cellular mediators in the peritoneal fluid at the beginning of the adhesion formation process. Among these mediators are IL-1a, TGF-a, EGF, TGF-b, IL–6 ve TNF-a. It is thought that IL-10 prevents adhesion (**14–17**). Moreover, the similarity of cytokine production between rats and humans in response to injuries to the peritoneum is emphasized (**18**).

The benefits of physical membrane barriers in preventing adhesions following surgical operations have been proved in several empirical studies (**19, 20**). Bioresorbable membrane (SeprafilmTM) comprising hyaluronidase and carboxymethyl cellulose is one of the substances which has the most significant efficiency. In the 1990s, following FDA approval, it has been used frequently in the USA. This material, which comprises hyaluronate and carboxymethyl cellulose, has a film-like structure, and its efficiency has been proved in many experimental studies (**21**). Although it is widespread for physical barriers to have positive effects to be used, it is difficult for adhesion barriers to be applied directly to the damaged surface. Physical barriers that are used, particularly during laparoscopic surgery, are limited (**22**).

Moreover, although bioresorbable membrane (SeprafilmTM) comprising hyaluronidase and carboxymethyl cellulose are not deemed to cause high financial burdens in developed countries and are evaluated to be cost-effective, it is not valid in most of the countries. It is also challenging to apply besides the fact that it is expensive.

The efficiency of Rapamycin has been connected primarily to the protection of cytokines during the inflammation phase, which is the first step in adhesion pathology and to the prevention of fibroblastic activity, which is the second step in the process following the inflammation phase. It is known that TGF-β increases adhesion formation significantly and that it causes surgical complications due to intensive adhesions has been shown that Rapamycin stops neovascularization by suppressing proinflammatory cytokines (**23, 24**).

The results of this study show that intraperitoneal application of Rapamycin, with its antiproliferative efficiency against T lymphocytes and selectively fibroblasts, reduces adhesion development significantly relative to the control group. No difference could be found between the control group and the group treated with SeprafilmTM. Although a statistically significant difference was not observed between the SeprafilmTM and Sirolimus group macroscopically and microscopically, it has been found to be meaningful for a statistically significant difference to be observed between the control group and the Sirolimus group. Under the scope of these results, and by keeping in mind that microscopic evaluation is a more objective evaluation in comparison to macroscopic evaluation, it can be said that the intraperitoneal usage of Rapamycin gave results that were superior to many agents that have been used until today in order to prevent adhesion formation.

CONCLUSIONS

Nowadays, the adhesion preventive effect of Sirolimus, which is frequently used in renal transplant patients, should be researched into as much as SeprafilmTM, and its usage as a combination with SeprafilmTM or on its own should be taken into consideration.

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REFERENCES

1. Risberg B. Adhesions: preventive strategies. The European journal of surgery Supplement : = Acta chirurgica Supplement. 1997;(577):32—39.

2. Herrick SE, Mutsaers SE, Ozua P, Sulaiman H, Omer A, Boulos P, et al. Human peritoneal adhesions are highly cellular, innervated, and vascularized. The Journal of pathology. 2000;192(1):67–72.

3. Müller SA, Treutner KH, Tietze L, Anurov M, Titkova S, Polivoda M, et al. Efficacy of adhesion prevention and impact on wound healing of intraperitoneal phospholipids. Journal of Surgical Research. 2001;96(1):68–74.

4. Erkol H, Perek S, Tunalı H, Sanal İ. Ratlarda karın içi yapışıkların önlenmesinde intraperitoneal heparin ve sodyum karboksimetilsellülozun etkileri. Çağdaş Cerrahi Dergisi. 1993;7:47–51.

5. DeCherney AH. Clinical problem of intraperitoneal postsurgical adhesion formation following general urgery and the use of adhesion prevention barriers. Surgical Clinics of North America. 1997;77(3):671–88.

6. Guvenal T, Cetin A, Ozdemir H, Yanar O, Kaya T. Prevention of postoperative adhesion formation in rat uterine horn model by nimesulide: a selective COX-2 inhibitor. Human Reproduction. 2001;16(8):1732–5.

7. Nair SK, Bhat IK, Aurora AL. Role of proteolytic enzyme in the prevention of postoperative intraperitoneal adhesions. Archives of surgery. 1974;108(6):849–53.

8. Conze J, Junge K, Klinge U, Weiss C, Polivoda M, Oettinger AP, et al. Intraabdominal adhesion formation of polypropylene mesh Influence of coverage of omentum and polyglactin. Surgical Endoscopy And Other Interventional Techniques. 2005;19(6):798–803.

9. Ellis H, Harrison W, Hugh TB. The healing of peritoneum under normal and pathological conditions. British journal of Surgery. 1965;52(6):471–6.

10. Tanphiphat C, Chittmlttrapap S, Prasopsunti K. Adhesive small bowel obstruction: a review of 321 cases in a Thai hospital. The American journal of surgery. 1987;154(3):283–7.

11. Perry Jr JF, Smith GA, Yonehiro EG. Intestinal obstruction caused by adhesions: a review of 388 cases. Annals of surgery. 1955;142(5):810.

12. Luijendijk RW, de Lange DC, Wauters CC, Hop WC, Duron JJ, Pailler JL, et al. Foreign material in postoperative adhesions. Annals of surgery. 1996;223(3):242.

13. Boys F. The prophylaxis of peritoneal adhesions: a review of the literature. Surgery. 1942;11(1):118–68.

14. Saba AA, Kaidi AA, Godziachvili V, Dombi GW, Dawe EJ, Libcke JH, et al. Effects of interleukin-6 and its neutralizing antibodies on peritoneal adhesion formation and wound healing. The American Surgeon. 1996;62(7):569–72.

15. Hershlag A, Otterness IG, Bliven ML, Diamond MP, Polan ML. The effect of interleukin-1 on adhesion formation. in the rat. American journal of obstetrics and gynecology. 1991;165(3):771–4.

16. Holschneider CH, Cristoforoni PM, Ghosh K, Punyasavatsut M, Abed E, Montz FJ. Endogenous versus exogenous IL-10 in postoperative intraperitoneal adhesion formation in a murine model. Journal of Surgical Research. 1997;70(2):138–43.

17. Cheong YC, Shelton JB, Laird SM, Richmond M, Kudesia G, Li TC, et al. IL-1, IL-6 and TNF-α concentrations in the peritoneal fluid of women with pelvic adhesions. Human Reproduction. 2002;17(1):69–75.

18. Golan A, Maymon R, Winograd I, Bukovsky I. Prevention of post-surgical adhesion formation using aspirin in a rodent model: a preliminary report. Human Reproduction. 1995;10(7):1797–800.

19. Rodgers KE, Johns DB, Girgis W, Campeau J. Reduction of adhesion formation with hyaluronic acid after peritoneal surgery in rabbits. Fertility and sterility. 1997;67(3):553–8.

20. Wiseman DM, Gottlick-Iarkowski L, Kamp L. Effect of different barriers of oxidized regenerated cellulose (ORC) on cecal and sidewall adhesions in the presence and absence of bleeding. Journal of Investigative Surgery. 1999;12(3):141–6.

21. Oncel M, Remzi FH, Senagore AJ, Connor JT, Fazio VW. Comparison of a novel liquid (Adcon-P®) and a sodium hyaluronate and carboxymethylcellulose membrane (SeprafilmTM) in postsurgical adhesion formation in a murine model. Diseases of the colon & rectum. 2003;46(2):187–91.

22. Zhou J, Elson C, Lee TD. Reduction in postoperative adhesion formation and re-formation after an abdominal operation with the use of N, O-carboxymethyl chitosan. Surgery. 2004;135(3):307–12.

23. Pozzetto U, Citterio F, Romagnoli J, Scatà MC, Nanni G. Effects of immunosuppressive regimens on plasma levels of transforming growth factor-beta 1 in renal transplant recipients. In: Transplantation proceedings. 2002. p. 1740–2.

24. Cao W, Mohacsi P, Shorthouse R, Pratt R, Morris RE. Effects of rapamycin on growth factor-stimulated vascular smooth muscle cell DNA synthesis inhibition of basic fibroblast growth factor and platelet-derived growth factor action and antagonism of rapamycin by FK506. Transplantation. 1995;59(3):390–4.