Original Article

Efficiency of sirolimus in prevention of adhesions around vascular grafts

M. Kanko, E. Ozbudak, A. Ozerdem, A. Aksoy, M. Kilic and K.T Berki

Departments of Cardiovascular Surgery, Kocaeli University School of Medicine, Kocaeli, Turkey

Request for reprints to Dr. Muhip KankoKocaeli Universitesi Tip Fakultesi, Kardiyovaskuler Cerrahi Ab.D., Kocaeli, Turkey,

E-mail: muhipkanko@yahoo.com

Abstract

*Background***:** Adhesions due to the reactions caused by the grafts used in the primary vascular operation can lead to various problems when a secondary operation is necessary. These problems include: bleeding, injuries to neighboring organs sand complications occurring due to a prolonged operation. We investigated the affects of sirolimus, which has antiproliferative effects on vascular adhesions.

Methods: The abdominal aortae of rats were explored and abrasions inflicted. Following the fixation of a PTFE (Polytetra floroetilen) graft on the abdominal aorta, rapamycin (sirolimus) was applied (in powder form) on the grafts of the study group. Four weeks later a laparotomy was done and the adhesions developed were evaluated.

Results: In the **study group** the adhesions were determined to be fewer in number and milder in severity. Severe adhesion were noted in the **control group**.

Conclusions: Therefore, sirolimus applied around the prosthesis in vascular operations, was determined to be effective in preventing possible adhesions.

Key words: Sirolimus(Rapamycin), adhesion,

Introduction

Adhesion formation is the physiological healing response of tissues against conditions such as infection and traumatic events. Adhesions developing as a result of primary vascular and cardiac operations cause an increase in the rate of morbidity and mortality during reoperations of these organs. The reason for this increase is the difficulties in the exposition of these organs in the secondary operation. The most frequent cause of adhesions occurring in vascular surgery is a result of the synthetic materials required to be employed. Tissue trauma, bleeding and infection play a significant role in the formation of adhesions. In order to prevent the formation of adhesions, studies have been conducted in which synthetic coating materials, anti-inflammatory, anti-mitotic, fibrinolytic and anticoagulant agents are used.[1,2] Rapamycin is an antibiotic I the macrolide group with immunosuppresive and anti-proliferative attributes. [3-6] Recently, it has been used in order to decrease the stent restenosis rate, especially in the coating of coronary and vascular stents. [7,8]The aim of this study, where PTFE was used as a vascular graft in a standard adhesion model, was to investigate the effects of sirolimus on the adhesions which may occur between this synthetic material and the surrounding tissues.

Note: In this study, rapamycin and sirolimus are used interchangeably

Material and Methods

This study was conducted in compliance with the guidelines of the National Institutes of Health Guidelines of Care and Use of Laboratory Animals, and approved by the Ethic Committee of Kocaeli University School of Medicine. In our study, 30 Wistar-Albino rats weighing between 200-300 gr. were used. All the animals were fasted 8 hours prior to the operation and fed by normal diet 6 hours post-operatively

Ketamine (40mg/kg) and Xylazine (10 mg/kg) were administered as anesthetics. Following the surgical debridement, a median laparotomy was performed to open the abdomen. Three randomized groups were formed consisting of 10 rats each.

First group (laparotomy group): Only the laparotomy was performed.

Second group (control group): Following the laparotomy, the retroperitoneum was opened and approximately 1.5 cm of abdominal aorta explored and an abrasion applied. A PTFE graft, 1x1 cm^2, was fixed with 7/0 non-absorbable monofilament polypropylene (Prolen, Ethicon) and the abdomen was closed with 3/0 absorbable braided monofilament polyglactin suture (Vycril, Ethicon).

Third group (Study group) (Sirolimus group): Following the laparotomy, 1.5 cm of the aorta was explored, the abrasion applied and a 1x1 cm^2 PTFE graft was fixed with 7/0 prolen. Then, 20 mg sirolimus (Rapamune, Wyeith) in powder form was applied inside and the abdomen was closed using 3/0 vycril. Following the application of these procedures, the test subjects were kept alive for eight (8) weeks post-operatively, and then a relaparotomy was performed with the same anesthetic protocol. Adhesions that formed between the PTFE graft, which was fixed on the aorta in the retroperitoneal region and in the surrounding tissues, were examined macroscopically. The formed adhesions were evaluated according to a Linsky[9] scale by an independent specialist who was not informed of the study groups. The total adhesion score was determined according to the degree of graft involvement of the adhesion, grade of vascularisation and the resistance of the adhesion (Table 1). The total score was calculated for each of the test subjects in the statistical assessment. The maximum total score was found to be 9 according to this scale.Statistical Analysis All the values are given as $\pm$ standard deviation. Kruskal-Wallis variance analysis and the Mann-Whitney U test were employed for statistical assessment. $P < 0.05$ is accepted as significant.

Results

Total adhesion score was determined to be 8,26 ± 0,9 in the control group (Table 2). Adhesion involvement percentages were determined to be significantly lower in the sirolimus group compared to the control group (p<0.05). No statistically significant difference was determined between the laparotomy group and the sirolimus group (p>0.05). While a high degree of adhesion was determined in the control group (group 2), the degree of adhesion in study group (group 3) was statistically significantly lower than the control group (p<0.05). The formed adhesions were visible between the intestinal system and other surrounding tissues and the graft. 80% of the study group test subjects either had no adhesion or only had grade 1 [15-16].

adhesions (Figure 1). 90% of the control group test subjects were found to have grade 3 adhesions (Figure2). The adhesions in the study group were of substantially thin and easily dissectable traits (Table 2).

Discussion

Reoperation rates in vascular surgery cases concerning the abdominal aorta is about 10%.[10] In vascular operations applied within the abdomen where a graft is used, aorta tissue or the surrounding tissues are placed around the graft. This way, an adhesion between the graft and the neighboring organs is attempted to be prevented. However, if there is no adequate surrounding tissue, adhesions develop between the neighboring organs and the graft. The morbidity and mortality rates during reoperations are generally due to complications associated with adhesions. These complications may include hemorrhages due to vascular injuries, the injury of neighboring organs in case the content of the injured organ is contaminated with infected material or bacterial contamination. Moreover, because the normal anatomical structure is disrupted due to adhesions, it becomes more difficult for the surgeon to perform the required dissection. Due to foreign body reactions occurring because of grafted material, adhesions develop more severely in cases with intraabdominal synthetic vascular grafts. Reoperations in such cases could have a course of high morbidity and mortality. Tissue adhesions associated with vascular surgery procedures do not include any specific symptoms. However, they may cause some gastrointestinal complaints. Adhesions developing following primary operations may lead to injuries in the neighboring organs or bleeding complications during reoperations. In order to investigate agents which can prevent adhesion formation, the mechanism of adhesion development should be examined in detail. Though adhesion formation is essentially a response to inflammation, factors such as prostaglandins occurring due to tissue trauma, chemotactic agents and tissue thromboplastin all contribute to adhesion formation. Hemorrhage, occurring due to tissue trauma or fibrinogen, occurring during exudation causes fibirin formation at the surgical field. Because fibrin has an adhesive property, it pulls neighboring tissues to itself and causes adhesion formation. In the recovery period, fibroblasts invade this fibrin mesh and by producing collagen fibers create fibrous connective tissue formation. Therefore, following the involvement of neoangiogenesis in this process, the adhesive tissue causes adhesion formation. [1,11-14]uring the adhesion formation process, fibroblast content increases in the 2nd week, followed by inclusion of vessel structures and connective tissue elements in the adhesion

Table I: Linsky scale is applied for determining adhesion score
      * The percentage of graft involvement in the adhesion.

Adhesion Percentage*	SCORE			
	0	1	2	3
No Adhesion	+			
*1- 25 %		+		
*26- 75%			+	
*76-100%				+
Adhesion severity				
No adhesion	+			
Filmy and avascular		+		
Moderate and filmy vascular			+	
Dense and significantly vascular				+
Adhesion grade(force ot separation)				
Grade 0: No adhesion	+			
Grade 1: No resistance		+		
Grade 2: Moderate resistance			+	
Grade3: Severe Resistance (Sharp dissection)				+

Table II: Adhesion scores of the groups.

Groups	Adhesion grade*			
	Adhesion involvement score	Adhesion severity score	Adhesion resistance score	Total Adhesion Score
Laparotomy	1,11± 0,20	1,12±0,49	0,50±0,3	2,73±0,25
Control	3,56±1,51 [b]	2,98±1,43 [c]	1,72±0,5[d]	8,26±1,21[a]
Study(Sirolimus)	1,08±0,6 [e]	1,01±0,9 [f]	1,01±0,4 [g]	3,10±0,6 [h]

* No significant difference was detected between control and sirolimus groups
Control vs laparatomy groups: [a] $p < 0.01$, [b] $p < 0.05$, [c] $p < 0.05$, [d] $p < 0.05$
Control vs sirolimus groups: [e] $p < 0.01$, [f] $p < 0.05$, [g] $p < 0.05$, [h] $p < 0.05$

Thus in approximately 3 weeks, the development of the adhesion becomes quite prominent. Studies for the evaluation of the degree of adhesion are usually held on in the first 10 weeks following induction. [17]In order to prevent possible adhesion formations occurring after surgical procedures, methods including the application of minimal trauma to tissue during surgery, inhibition of inflammation response and inhibition of coagulation are being performed. To prevent the formation of adhesions between the graft and the surrounding organs after the surgery, the synthetic grafts are covered with as much retroperitoneal tissue as possible. In spite of all these efforts, adhesions continue to form to matter in surgeries There is no agent for healing the already existing adhesion. However, various experiments and materials are employed to prevent or decrease the formation of adhesions. Among these agents are hyperosmolar saline, dextran, hyaluronic acid solution and recombinant tissue plasminogen activator. [18] A synthetic barrier, film or similar materials should prevent tissue inflammation, necrosis and tissue desiccation. These barriers should carry no infection risk and should not create a proper environment for fibroblast localization. Rapamycin, which we used in this study is an antibiotic belonging to the macrolide group with antifungal, immunosuppressive and **antitumoral attributes. Today, it is used in**

coatingcoronary and peripheric artery stents. Sirolimus coating of the stent is reported to prevent neointimal hyperplasia developing in the stent. [7,8] Also, sirolimus is used as an immunosuppressive agent on renal transplant agents. Due to its feature of in vitro and in vivo fibroplast proliferation from mesenchymal cells, we decided to use sirolimus in this study. [19-22] Rapamycin inhibits cell proliferation of connective tissue origin[23,24] by blocking the cell cycle in the G_1 and S phases, inhibiting cell proliferation. Additionally, sirolimus is reported to negatively affect wound healing There are studies reporting that it prevents intimal hyperplasia when applied topically around venous grafts[25] and that it also inhibits proliferation of smooth muscle cells and inflicts an anti-inflammatory effect on vessel walls. [26-30] Sirolimus inhibits the mitogenic affect of fetal calf serum in primary endothelial cell cultures and it is a potent inhibitor of cell proliferation mediated by growth factor. [23] It is not known which one of these ,

various affects is the main factor in adhesion formation, however, it is probable that it occurs as a result of their cumulative affect In some studies, sirolimus was applied topically around the vein grafts. These studies revealed a lower degree of neointimal hyperplasia in the venous grafts in the subjects who received topical sirolimus[25] In this study, the sirolimus applied may lead to the inhibition of intimal hyperplasia in especially the anastomosis area in the long term As this study reveals, sirolimus significantly decreases the adhesion formation between vascular grafts and peripherical tissues. Therefore, we believe further research is required on this subject both to empirically confirm our findings and to go beyond to explain the mechanisms of action. More detailed studies will contribute to the prevention and decrease of adhesi ons, which is an important problem for vascular surgeons

References

1. Gutmann J, Penzias A, Diamond MP. Adhesions in Reproductive Surgery. In: Wallach E, Zacur HI, editors. Reproductive Medicine and Surgery. Missouri: Mosby-Year Book, Inc.1995, p 691-93.
2. Whawell SA, Vipond MN, Scott-Coombes DM, et al. Plasminogen activator inhibitor-2 reduces peritoneal fibrinolytic activity in inflammation. Br J. Surg 1993;80:107-9.
3. Sehgal SN, Baker H, Vezina C. Rapamycin (AY-22,989), a new antifungal antibiotic. II. Fermentation, isolation, and characterization. J. Antibiotics 1975;28:727-31.
7. Sousa JE, Costa MA, Abizaid A, Lack of neointimal proliferation after implantation of sirolimus-coated stents in human coronary arteries: A quantitative coronary angiography and three-dimensional intravascular ultrasound study. Circulation 2001;103:192-5.
8. Sousa JE, Costa MA, Abizaid A, Sustained suppression of neointimal proliferation by sirolimus-eluting stens. One – year angiographic and intravascular ultrasound follow-up. Circulation 2001; 104:2007-11.
9. Linsky CB, Diamond MP,Cuninngham T,Adhesion reduction in a rabbit uterine horn model using an absorbable barrier, TC-7. J.Reprod Med 1987;32:17-20.
10. Naylor AR, Ah-See AK, Engeset J.Graft occlusion following aortofemoral bypass for peripheral ischaemia. Br J. Surg 1989;76:572-5
11. Holmdahl L. Making and covering of surgical footprints. Lancet 1999; May 1;353:1456-7.
12. Marc Hendrikx, Urbain Mees, Arthur C Hill,et al.Evaluation of a Novel Synthetic Sealant for Inhibition of Cardiac Adhesions and Clinical

4. Morris RE. Rapamycins: antifungal, antitumor, antiproliferative and immunosuppressive macrolides. Transplant Rev 1992;6:39-87.
5. Roque M, Reis ED, Cordon-Cardo C, et al. Effect of p27 deficiency and rapamycin on intimal hyperplasia: in vivo and in vitro studies using a p27 knockout mouse model. Lab Invest 2001;81:895.
6. Marx SO, Marks AR. The development of rapamycin and its application to stent restenosis. Circulation 2001;104:852-55.

Experience in Cardiac Surgery Procedures. Heart Surgery Forum 2001;4:204-10.
13. Becker JM, Dayton MT, Fazio VW, Prevention of postoperative abdominal adhesions by a sodium hyaluronate-based bioresorbable membrane: a prospective, randomized, double-blind multicenter study. J. Am Coll Surg 1996;183:297-306.
14. Bakkum EA, Van Blitterswijk CA, Dalmeijer RAJ, A Semiquantitative rat model for intraperitoneal postoperative adhesion formation. Gynecol Obstet Invest 1994;37: 99–105.
15. Buckman RF, Woods M, Sargent L, et al. A unifying pathogenetic mechanism in the etiology of intraperitoneal adhesions. J. Surg Res 1976 Jan;**20:1 -5**
16. **Holmdahl L, Al-Jabreen M, Xia G, et al. The impact of starch powdered gloves on the formation of adhesions in rats. Eur J. Surg 1994; 160:257-61.**
17. **Belluco C, Meggiolaro F, Pressato D. Prevention of postsurgical adhesions with an**

autocrosslinked hyaluronan derivative gel. J. Surg Res 2001;100:217–21.

18. Wiseman DM, Haung WJ, Johns DB, et al. Time-dependent effect of tolmetin sodium in rabbit uterine adhesion model. J. Invest Surg1994;7:527-32.

19. Kahan BD, Gibbons S, Tejpal N, Synergistic interactions of Cyclosporine and rapamycin to inhibit immune performances of normal human peripheral blood lymphocytes in vitro. Transplantation 1991;51: 232-9.

20. Tu Y, Stepkowski SM, ChouT-C, The synergistic effects of cyclosporine, sirolimus, and brequinar on heart allograft survival in mice. Transplantation1995; 59 :177-83.

21. Knight R, Ferraresso M, Serino F, Low-dose rapamycin potentiates the effects of subtherapeutic doses of cyclosporine to prolong renal allograft survival in the mongrel canine model. Transplantation 1993; Apr;55:947-9.

22. M Ferraresso, L Tian, R Ghobrial, Rapamycin inhibits production of cytotoxic but not noncytotoxic antibodies and preferentially activates T helper 2 cells that mediate long-term survival of heart allografts in rats. The Journal of Immunology 1994;153:3307-18.

23. Suren N Segal, Joseph S Camardo, Joseph A Scarola,et al. Rapamycin (Sirolimus, rapamune).Current Opinion In Nephrology And Hypertension 1995;4:482-7.

24. Molnar-Kimber KL, Rhoad A, Warner L,et al. Evidence that the anti-tumor and immunosupressive effects of rapamycin are mediated via similar mechanisms. Cold Spring Harbor Meeting on Cell Cycle, May 18-22 New York; Cold Spring Harbour Laboratory;1994:141

25. Thomas Schachner, Yping Zou, Alexander Oberhuber, et al. Local application of rapamycin inhibits neointimal hyperplasia in experimental vein grafts. Ann Thorac Surg 2004;77:1580-1585.

26. Schatz RA, Penn IM, Baim DS. Stent restenosis study (STRESS): analysis of in-hospital results [Abstract].Circulation 1993; 88 : I-594.

27. Roque M, Cordon-Cardo C, Fuster V. Modulation of apoptosis, proliferation, and p27 expression in a porcine coronary angioplasty model. Atherosclerosis 2000;153:315-22.

28. Suzuki T, Kopia G, Hayashi S-i, . Stent based delivery of sirolimus reduces neointimal formation in a porcine coronary model. Circulation 2001; 104: 1188-93.

29. Daiju Fukuda, Masataka Sata, Kimie Tanaka, et al. Potent Inhibitory Effect of Sirolimus on Circulating Vascular Progenitor Cells. Circulation 2005 111: 926 – 931

30. Braun-Dullaeus RC, Mann MJ, Dzau VJ. Cell cycle progression: new therapeutic target for vascular proliferative disease. Circulation 1998; 98: 82–89.

LEGENDS FOR ILLUSTRATIONS

Figure 1: The poor adhesion between the graft (arrow) and surrounding tissues of a test subject from the study group is evident

Figure 2: The dense adhesion and reactive tissue (grey arrow) arround of the graft (black arrow) a subject from the control group is evident