Erciyes Tıp Dergisi 12:469-477, 1990

EVALUATION OF DIFFERENT SETS OF TRAUMA ON ADHESION FORMATION IN RATS

Abdullah SAĞLAM*, Yaşar YEŞİLKAYA**

Summary: This Study was designed to examine the effects of the different types of trauma on adhesion formation in the rat uterine horn, the parietal peritoneum and the proximal colon. The adhesive effects of tissue ischemia, peritoneal stripping, crush injury, suturing peritoneal defects and anastomosis of the proximal colon were investigated.

The tissue ischemia was the most outstanding factor on adhesion formation among our adhesion models for the uterine horn and parietal peritoneum. The stripped peritoneal areas mostly heal without adhesion formation. Closure of the peritoneal defects may not be necessary for peritoneal healing and may also produce further adhesion formation. Many etiological factors such as ischemia, foreign body, infection were gathered in the colonic anastomosis and so adhesion formation rate in this model was perceived the highest of all groups.

Key Words: Peritoneal adhesions, wound healing.

Adhesions have become the most frequent cause of intestinal obstruction in recent years (1,20). Approximately one third of all intestinal obstructions in Western world are likely to be due to adhesions, constituting %60 of all small bowel obstructions (7). Since the end of the 19th century there has been great interest in the etiology and prevention of adhesions. In a review, mechanical trauma, tissue ischemia, thermal injury and foreign materials, and peritonitis of infectious origin are identified as being the most important factors behind adhesion formation (3). The aim of our study is to show the effect of different sets of trauma on adhesion formation.

Erciyes University School of Medicine, Department of General Surgery
**Assistant Professor of Surgery*
**Professor of Surgery*

Materials and Methods

A total of 87 adult syngeneic Wistar rats weighing 180-230 g was used in this investigation. Experimental studies were done on the peritoneal surface of the left and right uterine horns (Group UH), on the parietal peritoneum (Group PP), and on the proximal colon (Group PC). Each main group were also divided to three to asses the effects of peritoneal injuries, namely, ischemia, deperitonealization, and crushing or suturing of the peritoneum. All animals are operated under ether inhalation anesthesia and the abdomen was opened through midline laparatomy incision, postoperatively it was closed with two layer continuous 0.4 cat gut sutures.

Group UH1: 1.5 cm length of the left uterine horn devascularized by placing two No. 0.5 silk ligatures at its mesentery close against horn and then dividing the mesentery between ligatures. Same procedure were repeated on the left uterine horn. (20 injuries in 10 rats)

Group UH2: Same segments of the uterine horns were deperitonealized by stripping with scalpel. (18 injuries in 9 rats)

Group UH3: The 1.5 cm segment of the right uterine horn was crushed with an artery forceps placed for 5 minutes. The left uterine horn did not received any surgical trauma which was used for control of the group UH. (10 injuries in 10 rats)

Group PP1: Two small eminences of parietal peritoneum corresponding 1 cm square peritoneal area was lifted by an artery forceps and firmly ligated with 0.3 transfiction silk stitch at its base on left and right sides of the abdomen. By the way two buttons of peritoneum about 3 mm in diameter was made as an ischemic nodule for each rat. (18 injuries in 9 rats)

Group PP2: Parietal peritoneal defects each measuring 2 cm square were made on the left and right sides of the abdominal incision by stripping with scalpel (20 injuries in in 10 rats)

Group PP3: Similar peritoneal defects were made as in the group PP2 and these defects are closed with 0.4 cat gut running sutures (20 injuries in 10 rats)

Group PC1: 2 cm length of the colon just distal to ileocaecal valve of the colon is devascularized by placing ligatures at its mesentery and dividing its segmental vessels between these ligatures as in the group UH1. (9 injuries in 9 rats)

Group PC2: 2 cm length of same segment of the colon were stripped with scalpel. (10 injuries in 10 rats)

Group PC3: 1-2 cm of the colon distal to ileocaecal valve is resected and end to end anastomosis was performed as described before (31). (10 injuries in 10 rats)

All animals were killed by overdose ether anesthesia on the 8th postoperative day. The abdomen was explorated and adhesions at the trauma sites were graded according to "the adhesion scoring scale". (Table 1). Adhesion out of the trauma sites were not regarded. Fisher's exact Chi-square test were used for statistical evaluation.

Table I. Adhesion scoring scale

Grade 0	No macroscopic adhesions
Grade 1	Filmy adhesions those could be separated easily with blunt dissection without bleeding
Grade 2	Thick adhesions those could be separated by sharp dissection, or if separated blunt dissection cause some bleeding
Grade 3	Multiple adhesions at the same trauma site or one broad adhesion measuring 4 mm or more in its adhesive surface

Table II. Adhesion formation in the rat at 8'th postoperative day.(n shows the number of experimental injuries)

Site & Injury	Grade O		Grade 1	Grade 2	Grade 3	Total (Grade 1,2 & 3)	
Uterine Horn	n	%	n	n	n	n	%
İschemia (n:20)	3	15%	-	5	12	17	85%
Stripe (n:18)	14	78%	2	2	-	4	22%
Crush (n:10)	7	70%	2	-	1	3	30%
Parietal Peritoneum							
İschemia (n:18)	4	22%	3	2	9	14	78%
Stripe (n:20)	18	90%	1	1	-	2	10%
Sutur (n:20)	8	40%	3	3	6	12	60%
Proximal Colon							
İschemia (n:9)	2	22%	-	4	3	7	78%
Stripe (n:10)	8	80%	-	2	-	2	20%
Anastomosis (n:9)	0	0%	-	5	4	9	100%

Table III. İstatistical comparison of the groups. (*p<0.01 and **p<0.05 significant, *** p>0.05 no significance)

Uterine Horn		Parietal Peritoneum		Proximal Colon	
Trauma	p Value	Trauma	p Value	Trauma	p Value
Ischemia-Stripe	0.00012*	Ischemia-Stripe	0.00003*	Ischemia-Stripe	0.022**
Ischemia-Crush	0.0048*	Ischemia-Sutur	0.204***	Ischemia-Anastomosis	0.253***
Stripe-Crush	0.819***	Stripe-Sutur	0.00109*	Stripe-Anastomosis	0.023**

Results

The results are summarized in Table II. All animals, except one in the colonic anastomosis group, tolerated surgery well. Only one or sometimes two wound infection was experienced in each group.

When group UH1 rats were evaluated, 17 of the uterine horns were found to have grade 2 and 3 adhesions while 3 were free of adhesion. Most of adhesions were in form of mesenteric attachments to the ischemic uterine horns. There was a case with conglomeration of the small intestinal loops and the omentum around the ischemic uterine segment which was also valued as grade 3. In the uterine horn peritoneum stripped group (Group UH2) there were 4 grade 1 and 2 adhesions the others in this group were free of adhesions. Similarly in the uterine horn crush injury and control group (Group UH3) there were 3 adhesions on the right side, and no adhesion on the left side (control side). Two of the adhesions formed in this group were grade 1 type. In one rat there were adhesions between injured uterine segment and multiple intestinal loops, as grade 3.

Studies on the parietal peritoneum gave results similar to uterine horn peritoneum, in the ischemic parietal peritoneum group (Group PP1) there were 14 grade 1 to grade 3 adhesions. Only 4 of the ischemic parietal nodules were free of adhesions. The stripped parietal peritoneum caused only two filmy adhesions in Group PP2. If the abrasive surface of the the parietal peritoneum closed by approximating the wound edges we had observed 12 adhesions out of 20 insults in this group with 1 to grade 3 (Group PP3).

Seven of the 9 ischemic colons showed adhesions(Group PC1), four of them were grade 2 and the remaining 3 were grade 3 adhesions. Deperitonealization of the visceral peritoneum in group PC2 caused adhesions only in two of the cases. All of the colonic anastomoses produced adhesions (Group PC3) every anastomotic line was covered by mesenteric fat. In two of the cases great omentum was also involved in adhesion formation. Other two anastomoses were covered by a conglomeration of the small intestine and omentum. In this

group separation of the formed adhesions always resulted with some bleeding though all they were graded grade 2 and grade 3.

We have seen only a few adhesions in the abdomen other than trauma sites, which were mostly between the omentum and the undersurface of the anterior abdominal incision.

Discussion

Mechanical trauma, infections, tissue ischemia, thermal injury and foreign material are the main etiological factors that induce adhesion formation. In this study we have investigated the effects of different types of mechanical trauma and tissue ischemia. It's well known that healing process begins within 24 hours of the injury and is generally completed by the 8th postoperative day so we have prefered the 8th day to examine postoperative adhesion formation (10,11,27,29,30,40).

Adhesion formation begins by the development of fibrinous network. Trauma or inflammation produces an outpouring of fibrinogen which form fibrin clots. These fibrin clots causes adherence of the adjacent structures. The fibrinous attachments naturally disappear by fibrinolysins released from mesothelial cells (6,14). Plasminogen activator activity (PAA) converts the plasminogen to plasmin which has the fibrinolytic activitiy (22). Raftery had been documented in his studies that mesothelial cells have PAA (25,26,28). If there is a deficit in local PAA developed fibrinous network will be invaded by fibroblasts and become organized into fibrous tissue. Mesothelial defects has impaired PAA and this activity returns to normal by mesothelial healing. During the first 48 hours of healing mesothelial defects fibrinolytic activity is absent but, thereafter it increases to greater than normal (25,26,28,35). It has been also shown that adhesion formation could be prevented by adding recombinant PAA (14).

İschemic injury of the parietal and visceral peritoneum was resulted adhesion formation with high frequency in all three groups (Table 1). In the literature it is shown that peritoneal ischemic injury is a strong stimulus for adhesion formation. (8,9,23,34,35). The mechanism of ischemia induced adhesion were studied and it is found that tissue ischemia, and low tissue oxygen tension results fibroblast proliferation (13), and ischemic tissue seems to have impaired fibrinolytic activity (4) ischemia results loss of the mesothelial surface (2). Ellis also showed that adhesions those develop in relation to areas of ischemia represent vascular growths into such ischemic tissue (8).

The other injury that is common for all three groups is deperitonealization which was studied both on parietal peritoneum (Group PP2) and visceral peritoneum (Groups UH2, PC2). Our results are similar for all three groups; stripped peritoneal surfaces rarely cause adhesion formation. Many other investigestions have showed that peritoneal defects heal without adhesion formation (8,9,19). In fact loss of the peritoneal surface mesothelial cells results impaired tissue PAA, but if the neighboring peritoneal surface is intact this loss of PAA may be tolerated. Though we rarely see adhesions at the site of peritoneal defects. These findings now have forced many investigators to use new adhesion models that produce kissing peritoneal defects (19,22).

In Group UH3 we see 3 adhesions(33%). This group adhesions may be caused partially tissue ischemia that has been produced by crush injury of tissues and partially by the loss of peritoneal mesothelial cells those have PAA.

In most tissues defects heal by filling of the defect base with non-specific fibrous tissue and surface restoration by epithelium advancing from the wound margins. For this reason wound edges are brought into apposition for obtaining primary healing. There is ample evidence in the literature that peritoneal healing differs distinctly from the healing of other tissues. There are four major theories on the mechanism of peritoneal healing:

1. Mature mesothelial cells from adjacent surfaces multiply shed and repopulate the site of injury (39,40)

2. Replenishing cells originate from bone marrow (28).

3. Subperitoneal steam cells transform into mesothelial cells (24,27).

4. Free floating serosal cells in the peritoneal fluid settle on the injured surface (15,29). Although repair mechanism controversial there is agreement that the time required for the complete healing of small and large areas of the peritoneum is similar and the healing is complete within 8 days. Also it seems quite possible that some of the above-mentioned processes work together (10,11).

As we see in group UH2, PP2 and PC2 loss of the peritoneal surface generally does not result with adhesion formation, but if these defects are closed by suturing or stapling as in Group PP3 one will observe much more adhesion formation (8,12,18,19,33). These observations led investigators to find an answer to "Whether the peritoneum need to be closed at laparatomy incisions". In two trials it's found that leaving or closing peritoneum as a separate layer in laparatomy wound makes any noticeable difference in the incidence of adhesion formation (5,16). We understood that closing peritoneal defects will not preclude adhesion formation but may cause to much more adhesion as in our study. Suturing of the peritoneal defects renders tissues ischemic and though cause adhesion formation. Tension across the suture line can also stimulate adhesion formation (7,18).

Stripping of the peritoneum from serosal surfaces of the intestines does not cause adhesion formation unless it's not too deep to the mucosa (Group PC3). Same repair mechanism work for mesothelial surfaces so healing of the visceral and parietal peritoneum must be the same, but, if one strips all the layers leaving the mucosa bare, he will experience adhesion formation, because vascular supply of the mucosa will be abolished and the mucosa will be rendered ischemic (Unpublished Data).

Anastomosis of the colon almost invariably produces adhesion formation (7,41). Many factors may be responsible the from adhesion formation in this model; tissue ischemia will occur from anastomotic sutures, there will be fecal foreign contamination and infection at the site of anastomosis and lastly there may be also some peritoneal injury. It is shown that if the foreign material and serosal injury are to gether they will cause much more adhesions than every each other does (21,32,37). That may be the reason why mechanical intestinal obstructions due to adhesions are much more common after large bowel surgical procedures if compared to the other abdominal operations.

Conclusion

Since the resected peritoneal areas heals completely and had minimal adhesion formation reperitonealization may not be necessary. Also closure of the peritoneum as a separate

layer appears to be not necessary for peritoneal healing. Adhesion formation can be reduced by meticulous surgical technique, and this must include, prevention of tissues from crushing, and not to leave much more ischemic tissue beyond hemostatic ligatures. The peritoneum also should be prevented from any foreign material and also any fecal contamination, for preventing infection as well as adhesions.

References

1. Bevan PG: Adhesive obstruction. **Ann R Col Surg Eng** 66: 164-169,1984.
2. Booth WV, Zimny M, Kufman HJ, Cohn I: Scanning electron mcroscopy of small bowel strangulation obstruction . **Am J Surg** 125: 129-33, 1973.
3. Breland U, Bengmark S: Peritoneum and adhesion formation. In Bengmark S(Ed): **The Peritoneum and Peritoneal Access.** Butterworth, Cambridge 1989, pp 122-9.
4. Buckman RF, Woods M, Sargent L, Gervin AS: A unifying pathogenetic mechanism in the etiology of intraperitoneal adhesions. **J Surg Res** 20: 1-, 1976.
5. Ellis H, Heddle R: Does the peritoneum need to be closed at laparatomy. **Br J Surg** 64: 733-736, 1977.
6. Ellis H: İnternal overhealing. The problem of intraperitoneal adhesions **World J Surg** 4: 303,6, 1980.
7. Ellis H: İntestinal adhesions. **Ann Chir Gyn** 72: 237-8, 1983.
8. Ellis H: The aetiology of post-operative abdominal adhesions. An experimental study. **Br J Surg** 50: 10-6 1962.
9. Ellis H: The causes and prevention of intestinal adhesions. **Br J Surg** 69:241-3 1982.
10. Eskeland G, Kjaerheim A: Regeneration of parietal peritoneum in rats. An electron Microscopical study. **Act Path et Microbiol Scandinav** 68: 379-95, 1966.
11. Eskeland G: Regeneration of parietal peritoneum in rats. A light microscopical study. **Act Path et Microbiol Scandinav** 68: 355-78, 1966.
12. Glucksman DL: Cirrhosal integrity and intestinal adhesions. **Surgery** 60:1009-11, 1960.
13. Heughan C, Zederfeldt BH, Grislis G Hunt TK: Effect of dextran solutions on oxygen transport in wound tissue. **Acta Chir Scand** 138: 639-, 1972.
14. Holtz G: Prevention of postoperative adhesions. **J Repr Med** 24: 141-6, 1980.
15. Johnson FR, Whitting HW: Repair of parietal peritoneum. **Br J Surg** 49: 653-60, 1962.
16. Karipineni RC, Wilk PJ, Danese CA: The role of the peritoneum in the healing of abdominal incisions. **Surg Gynecol Obstet** 142: 729-30,1976.
17. Leach RE, Raymond HL: Reduction of postoperative adhesions in the rat uterine horn model with poloxamer 407. **Am J Obstet Gynecol**162:1317-9.

18. Lindenberg S, SetntoftP, Sorensen SS, Olesen HP: Studies on prevention of intra-abdominal adhesion formation by fibrin sealant. **Acta Chir Scand** 151:525-7,1985.
19. McDonald MN, Elkins TE, Wortham GF, Stoval TG, Ling FW, McNeeley SG: Adhesion formation and Prevention after peritoneal injury and repair in the rabbit. **J Reprod Med** 33: 436-9, 1988.
20. McEntee G, Pender D, Mulvin D, McCullough M, Naeeder S, Farah S, Badurdeen MS, Ferraro V, Cham C, Gillham N, Matthews P: Current spectrum of intestinal obstruction. **Br J Surg** 74: 976-80,1987.
21. McEntee GP, Stuart RC, Byrne PJ, Leen E, Hennessy TP: Experimental study of starch induced intraperitoneal adhesions. **Br J Surg** 77: 1113-4, 1990.
22. Menzies D, Ellis H: Intra-abdominal adhesions and their prevention by topical tissue plasminogen activator. **J R Soc Med** 82: 534-5, 1989.
23. Nishimura K, Nakamura RM, DiZerega GS: Biochemical evaluation of postsurgical wound repair: Prevention of intraperitoneal adhesion formation with ibuprofen. **J Surg Res** 34: 219-26, 1983.
24. Raftery AT: Regeneration of parietal visual peritoneum. An electronmicroscopical study. **Br J Surg** 60:293-9, 1973.
25. Raftery AT: Effect of peritoneal trauma on peritoneal fibrinolytic activity and intraperitoneal adhesion formation. **Eur Surg Res** 13:397-401, 1981.
26. Raftery AT: Method for measuring fibrinolytic activity in a single layer of cells. **J Clin Pathol** 34: 625-9, 1981.
27. Raftery AT: Regeneration of parietal and visceral peritoneum. A light microscopical study. **Br J Surg** 60:293-9, 1973.
28. Raftery AT: Regeneration of peritoneum: A fibrinolytic study. **J Anat** 129:659-64, 1979.
29. Ryan GB, Grobety J, Majno G: Mesothelial injury and Recovery. **Am J Pathol** 71:93-112, 1973.
30. Ryan GB, Grobety J, Majno G: Postoperative peritoneal adhesions. **Am J Path** 65:117-148,1971.
31. Sağlam A, Bengisu N: Intestinal anastomosis without angulation. **Erciyes Medical Journal** (In press).
32. Sağlam A: Starch induced peritoneal adhesions. **Br J Surg** (Accepted for the correspondence column) 1991.
33. Singleton AO, Rowe WB, Moore RM: Failure of reperitonealization to prevent abdominal adhesion in the dog. **Am Surg** 18: 789-92, 1952.
34. Steinleitner A, Lambert H, Kazensky C, Sanchez I, Sueldo C: Reduction of primary postoperative adhesion formation under calcium channel blockade in the rabbit. J **Surg Res** 48: 42-5, 1990.
35. Thompson JN, Paterson-Brown S, Harbourne T, Whawell SA, Kalodiki E Dudley HAF: Reduced human peritoneal plasminogen activating activity: Possible mechanism of adhesion formation. **Br J Surg** 76: 382-4, 1989.

36. Steinleitner A, Lambert H, Montoro L, Kelly E, Swanson J, Sueldo C: The use of calcium channel blockade for the prevention of postoperative adhesion formation. **Fertil Steril** 50:818-21, 1988.

37. Tolhurst Cleaver CL, Hopkins AD, Kee Kwong KCNG, Raftery AT: The effect of postoperative peritoneal lavage on survival, peritoneal wound healing and adhesion formation following fecal peritonitis: An experimental study in the rat. **Br J Surg** 61:601-4, 1974

38. Wagner JC, Johnson NF, Brown DG, Wagner MMF: Histology and ultrastructure of serially transplanted rat mesotheliomas. **Br J Cancer** 46: 294-9, 1982.

39. Watters WB, Buck RC: Scanning electron microscopy of mesothelial regeneration in rats. **Lab Invest** 26: 604-9, 1972.

40. Whitaker D, Papadimitriou J: Mesothelial healing: Morphological and kinetic investigations. **J Pathol** 145: 159-75, 1985.

41. Young HL, Wheeler MH, Morse D: The effect of intravenous aprotinin (Trasylol) on intraperitoneal adhesion formation in the rat. **Br J Surg** 68: 59-60, 1981.