

# An experimental study on the etiology of adhesion formation

Nusret AKYÜREK, Mustafa TERCAN

Dept. of General Surgery, Medical School of Erciyes University, Kayseri, TURKEY

*We compared the sterilized rat feces with glove powder in order to test the effect of foreign bodies on the adhesion formation. We studied the role of infection by using fresh rat feces. 90 rats weighting 200 to 250 g were included in this study and they were divided into 12 groups. Laparotomy was performed in one group as a single procedure. Glove powder suspension (GP) was studied in three groups as low, high and very high concentrations (HGP). Sterile and fresh rat feces were studied in two groups; without abrasion (SRF, FRF) and with abrasion (Ab+SRF, Ab+FRF). 10 models 5 rats with abrasion and 10 models were formed from 10 rats without abrasion. In the 14th postoperative day laparotomy was repeated and the adhesions to the abrasion site in the groups with abrasion and to the inferior part of the liver in the others were evaluated. Adhesion was not seen in laparotomy group, abrasion group, the groups of low concentration glove powder suspension with and without abrasion, and the group of high concentration glove powder suspension without abrasion. The group of very high concentration glove powder suspension was compared with the group of sterile rat feces for the foreign body effects. Also this foreign body effect of sterile rat feces was compared with the infection and foreign body effect of fresh rat feces on the adhesion formation. The adhesion score was  $0.8 \pm 0.8$  in the HGP group without abrasion,  $1.5 \pm 0.6$  in the Ab+HGP group,  $0.4 \pm 0.7$  in FRF group,  $0.6 \pm 0.8$  in SRF group,  $2.2 \pm 1.0$  in Ab+FRF group and  $2.2 \pm 1.0$  in Ab+SRF group. The comparison of SRF, FRF and HGP groups and the comparison of Ab+SRF and Ab+FRF groups yielded no statistical difference in respect to the adhesion scores ( $p > 0.05$ ). Adhesion score was significantly high in Ab+FRF and Ab+SRF groups in comparison to Ab-HGP group ( $p < 0.05$ ). As a result we found that the rat feces produced adhesion as a foreign body and that the rate of adhesion formation increased in case of peritoneal surface loss. [Turk J Med Res 1994; 12(3): 97-102]*

Key Words: Abdominal surgery, Adhesion, Rats

Intraabdominal adhesions after the abdominal surgical procedures are still a common problem. Followings are important in the prevention of adhesion; (1) homeostasis, (2) avoidance of too much manipulation of the abdomen (3) avoidance of serosal defect formation and the repair of formed serosal defect, (4) clearance of glove powder, and (5) avoidance of the use of surgical materials such as surgical gel foam. Adhesion may form in spite of these precautions. The most important cause of small intestinal obstructions is postoperative adhesions (1,2). Peritoneum normally has a physiological mechanism preventing adhesion (1). If peritoneal surface is damaged, vasoactive amine is

secreted and the permeability of vessels increases. The repair mechanism is activated in this site. The first cell group seen here is fibroblasts and primitive mesenchymal cells. The repair activity is normally performed by mesothelial cells, but excessive fibrin deposition in the adhesion is followed by fibrosis (1,2). Intraabdominal adhesions are often formed and the complications develop due to these adhesions after the emergency surgical procedures related to colorectal region and the acute appendicitis. The high bacterial content of the feces is thought to be responsible for these complications, but the role of feces on the adhesion formation after these operations may be due to the foreign body effect. We did not see any study in the literature investigating the foreign body effect of the feces on the adhesion formation.

We studied the foreign body effect of sterile rat feces on the adhesion formation by comparing it with laparotomy, abrasion, fresh rat feces with and without abrasion and glove powder in various concentrations.

Received: Jan. 12, 1994

Accepted: April 6, 1994

Correspondence: Nusret AKYUREK

Dept. of General Surgery,  
Medical School of Erciyes University,  
Kayseri, TURKEY

## MATERIALS AND METHODS

We used a total of 90 Wistar Albino rats from both sexes weighting 200 to 250 g. The rats were anesthetized with ether. The abdominal skin was shaved and cleaned with povidone-iodine solution and then laparotomy was performed by a midline incision. Sterile techniques were used during the surgical procedures. The rats were divided into 12 groups (Table 1). 10 rats in the laparotomy group were only explored by hands and the procedure finished. 5 rats were used in the only abrasion group. Abrasions were done bilaterally in the size of 1.5x2 cm, 1 cm away from the midline incision by rubbing peritoneum 10 times with a hand drill (Ae Sculap CD 9, FR-47, 8 mm Spherical Burrs, Germany) (Fig 1). So two abrasion models were formed on one rat.

Glove powder suspension was prepared from the gloves used during operations. Every glove was checked by alcohol iodine test for the presence of powder. Three different concentrations in which the amount of foreign body was compared were sampled in glove powder groups. High concentration glove powder suspension was prepared by washing five pairs of gloves in 250 ml physiological saline and low concentration glove powder suspension by washing the former pairs of gloves again in 250 ml physiological saline. We administered 125 mg glove powder intraperitoneally in very low concentration GP group and 250 mg glove powder in high concentration GP group. Very high concentration glove powder suspension (HGP) was prepared by diluting 1 g. glove powder in 3 ml physiological saline. We used the glove powder suspensions with and without abrasion. 10 models were formed from 10 rats in the groups of low, high and very high glove powder concentration without abrasion and 10 models were formed from 5 rats in the groups with abrasion. Then 3 ml of prepared suspension was given intraperitoneally to each animal. Intraperitoneal sterility was confirmed by culture before starting each experiment.

Sterile rat feces was prepared in the following manner: 4 g of rat feces sterilized by ethylene oxide to avoid the effect of heat and other factors was dissolved in 100 ml physiological saline mechanically. Then it was filtered through a double layer of veil. Sterile rat feces suspension (SRF) was used with and without abrasion. 10 models were formed from 10 rats in the SRF group and 10 models from 5 rats in the group of SRF with abrasion. 3 ml of SRF suspension (approximately 0.120 g) were given intraperitoneally to each rat. The amount of sterile rat feces was almost the same with that of low concentration glove powder suspension (0.125 g). Before the administration SRF was inoculated into the culture media and it was observed that there was no growth.

The purpose in the fresh rat suspension group was the formation of peritonitis and still prevent the death of the rats. So the following suspension was used after some investigations.

Fresh rat feces suspension was prepared as follows: 4 g of fresh rat feces was crushed in 12 ml normal saline contained in Erlen-Mayer by glass baget in sterile conditions and a suspension was produced. This suspension was filtered through a double layer of veil. Filtered material was diluted in the ratio of 1:2. 1/10, 1/10<sup>2</sup>...1/10<sup>5</sup> dilutions were prepared and colonies were counted. Three plaques were used for each dilution (3). Bacteria were evaluated by the standard laboratory methods (4). This fresh rat feces suspension (FRF) was used with and without abrasion. 10 models were formed from 10 rats in the group without abrasion (FRF group) and 10 models were formed from 5 rats in the group with abrasion (Ab+FRF group). Then 1 ml/kg of FRF was given intraperitoneally to each rat and from the same solution the bacterial species were determined and counted by culture (5). In the culture we observed a flora (mean values) consisting of 6x10<sup>7</sup>/ml enterococci, 1.5x10<sup>7</sup>/ml staphylococci, 4x10<sup>5</sup>/ml proteus and 4x10<sup>5</sup>/ml bacteroides.

Table 1. Study groups and model numbers

GBLudy model	Rat number	Model number	The amount administered
1	Laparotomy	10	10
2	Abrasion	5	10
3	Low GP without abrasion	10	10
4	Low GP with abrasion	5	10
5	High GP without abrasion	10	10
6	High GP with abrasion	5	10
7	Very high GP without abrasion	10	10
8	Very high GP with abrasion	5	10
9	FRF without abrasion	10	0.20-0.25 ml
10	FRF with abrasion	5	0.20-0.25 ml
11	SRF without abrasion	10	3 ml (0.120 g)
12	SRF with abrasion	5	3 ml (0.120 g)
Total		90	120

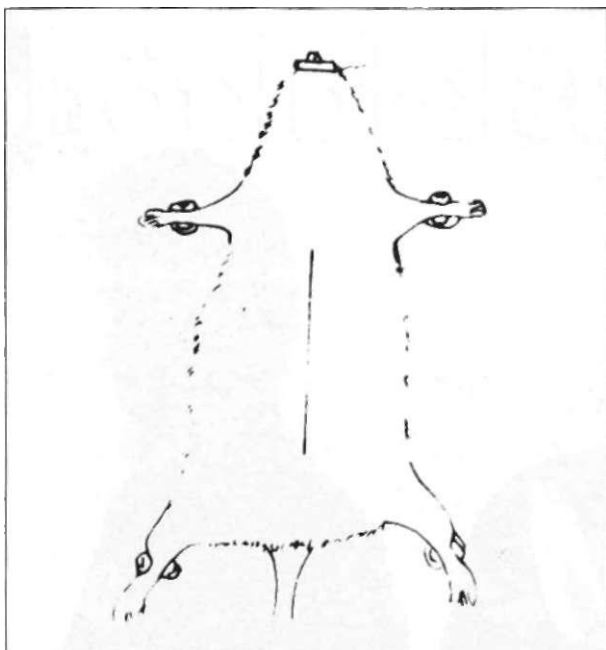


Figure 1. The appearance of abrasion model in the anterior abdominal wall.

Laparotomy incisions were closed by 3/0 chrome catgut suture. All of the rats were sacrificed in the 14th day by excessive ether anesthesia and laparotomy was repeated through the same incision site. The presence and the extend of adhesions were searched. The presence of intraperitoneal infections were evaluated by culturing peritoneal fluid from FRF group.

For the evaluation of adhesions in the groups with abrasion the grading system of Linsky et al (6) according to the size and severity of the adhesions was used.

Grade 0 : No adhesion.

Grade 1 : 25% adhesion in the abrasion area.

Grade 2 : 50% adhesion in the abrasion area.

Grade 3 : Adhesion in all of the abrasion area.

There is another scoring for the separation of adhesions:

Grade 0.0 : No resistance to the separation of adhesions.

Grade 0.5 : Mild resistance to the separation of adhesions.

Grade 1 : Need for cutting for the separation of adhesions.

The evaluation of adhesions in the groups without abrasion was done in a similar way by recording the size and severity of the adhesions to the inferior surface of the liver, the stomach, the intestine and the omentum. A total adhesion score ranging from 0.0 to 4.0 was formed by adding two grading scales according to the size and severity of adhesions. Student t test was used for the statistical analysis.

## RESULTS

The number of adhesion models and adhesion scores were listed in Table 2. No adhesion was seen in the laparotomy group, the abrasion group, the groups of low and high concentration GP with abrasion, but there were adhesions in varying degrees in the other groups (Fig. 2,3). Adhesion was also seen in one model (10%) in the group of high concentration GP with abrasion. We found the adhesion scores (mean±SD) as 0.8±0.8 (in 5 of 10 models, 50%) in HGP group, 1.5±0.6 (in 9 of 10 models, 90%) in Ab+HGP group, 2.2±1.0 (in 9 of 10 models, 90%) in Ab+SRF group, 0.4±0.7 (in 3 of 10 models, 30%) in FRF group and 2.2±1.0 (in 9 of 10 models, 90%) in Ab+FRF group. The highest adhesion scores were seen in Ab+FRF and Ab+SRF groups.

Adhesion scores were compared between the groups with and without abrasion (Table 3). There was no significant difference between Ab+HGP and SRF groups, Ab+HGP and FRF groups and FRF and SRF groups ( $p>0.05$ ). The adhesion score of Ab+FRF

Table 2. The adhesion scores in the study groups

Groups	Model no	No. of Rats	No. of models with adhesion	%	Adhesion score/model Mean±SD
HGP	10	10	5	50	0.8±0.8
Ab+HGP	10	5	9	90	1.5±0.6
FRF	10	10	3	30	0.4±0.7
Ab+FRF	10	11	9	90	2.2±1.0
SRF	10	10	4	40	0.6±0.8
Ab+SRF	10	5	9	90	2.2±1.0
Total	60	45	39		

Ab      Abrasion  
HGP     Sterile rat feces  
FRF     Fresh rat feces

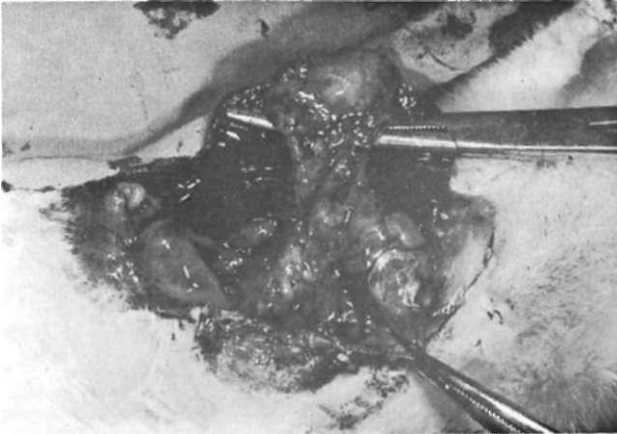


Figure 2. The adhesion of omentum on which granulomas are formed to the inferior surface of liver in the model without abrasion.

group was significantly higher than that of Ab+HGP group ( $p<0.05$ ) whereas there was no significant difference between the adhesion scores of Ab+FRF and Ab+SRF groups ( $p>0.05$ ). It was found that the adhesion score of Ab+SRF group was significantly higher than that of Ab+HGP group ( $p<0.05$ ).

We found tumor-like granulomas in the SRF, FRF and HGP groups. They were seen to be starch powder granulomas and fibrotic structures containing purulent material composed of feces when they were cut.

Proteus grew in one case in Eosin-Methylene-Blue (EMB-Oxoid) broth and proteus in two cases, proteus and staphylococcus in one case in the blood agar in FRF group after relaparotomy. Only enterococcus grew in the blood agar in the group with abrasion.

## DISCUSSION

It was observed in the postmortem studies and laparotomies that the development of postoperative adhesions was quite common. The causative factors include mechanical trauma, venous stasis, ischemia and bacterial contamination most commonly (7). Weibel

Table 3. The comparison of adhesion scores in which the adhesion formation was observed

Groups		t	P
HGP	SRF	1.0	0.343
HGP	FRF	1.5	0.168
FRF	SRF	1.0	0.343
Ab+SRF	Ab+HGP	4.0	0.003
Ab+SRF	Ab+FRF	0.6	0.591
Ab+FRF	Ab+HGP	4.0	0.003

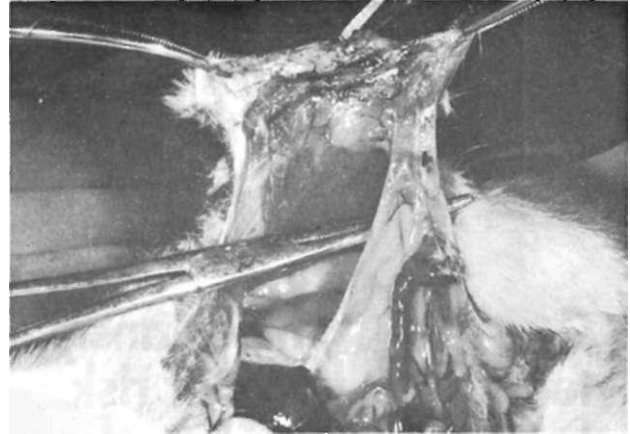


Figure 3. The adhesion of intraperitoneal organs to the area of peritoneal defect formed on the anterior abdominal wall in the model with abrasion.

and Majno (8) reported in the postmortem study of 298 cases that 67% of those undergone laparotomy once and 93% of those undergone multiple laparotomies were found to have adhesions. Fifty five of those having adhesions were examined histopathologically and foreign body granulomas were detected in 66%. In another study the rate of adhesion formation after laparotomy was found to be more than 44% (9). Fibrin can not be dissolved and subsequently adhesions are formed because of prevention of plasminogen activation on the peritoneal surface due to infections, trauma, foreign bodies and ischemia. If the sequence of these events continues a few days, peritoneal adhesions develop due to fibroblastic reactions (10). Holtz and Ellis (10,11) suggested the role of foreign body on the peritoneal adhesion formation besides the deficiency of fibronolysis due to ischemia and mechanical trauma. Peritoneal blood vessels consist of collagen, fibroblast, lymphocyte, plasma cell, mast cells and fat cells. Peritoneal adhesions are due to fibroproliferative inflammation of mesothelial tissue after abdominal surgery (10). Vasoactive substances such as histamine, chemotactic factors and growth factors are synthesized and secreted from the peritoneum in the inflammation area. The vascular permeability increases and the transudation of peritoneal cells occurs. There is an important role of arachidonic acid metabolites on this response too. It is known that the activity of plasminogen increases in the area of peritoneal defect. The balance is disturbed between fibrin activity and fibrinolytic activity during adhesion formation. Here the balance is against the fibrinolytic activity. Fibrin stabilization is achieved in the 3rd day during adhesion formation, i.e. adhesion formation starts in the 3rd day, becomes visible macroscopically in the 8th to 10th days and can be examined histologically in the 3rd week. The intraperitoneal foreign

bodies stimulate inflammatory vascular response of the adjacent structures. Capperauld (12) showed that the starch powder in the glove started to be absorbed from the intraperitoneal cavity in the animals in 48 hours, but the masses of starch powder insisted 70 days or more. In another study it was reported that glove starch powder was absorbed slowly from the intraperitoneal cavity and this absorption continued for 6 months (13). We saw granulomas of starch powder in the groups in which starch powder was used in the relaparotomies performed 2 weeks later. We didn't find any study on the absorption of feces. We found that the feces was not absorbed when we cut the granulomas. Prednisone is known to decrease the histological reaction due to the powder (14). Glove powder, during laparotomy and intestinal contents and suture materials during intestinal operations may lead to the intraabdominal adhesions and granulomas. It was recorded that the glove starch powder caused adhesion formation in a rate of 60%. In these studies the duration of adhesion formation, i.e. the formation of bands after granulomas due to glove powder was seen to be 5.5 months postoperatively (13). We noted adhesion formation in 15 (25%) of 60 models in the groups in which glove powder was used in varying concentrations. This result is lower than the other records (13,15).

Adhesion was formed only in HGP groups in our study. Mc Entee et al (16) calculated that 42 mg starch powder was given to each rat when the laparotomies were performed with the suspension prepared by washing five pairs of surgical gloves once. The adhesion rate after 8-10 weeks was 76% in these rats. We noted adhesion formation only in the HGP group (70%) in which we administered 1 g starch powder to each rat. Eldegez et al (15) recorded the adhesion rate as 50% in the study in which they administered 1 g starch powder to each rat. So the amount of starch powder has an important role in the adhesion formation. Prominent starch granulomas and the bands related to these granulomas were seen between the intraabdominal organs in the HGP group in our study. Mc Entee et al (16) suggested that 21 g of glove powder was left in the abdomen when the unwashed gloves were used during the surgery. We also saw that the high concentration glove powder with abrasion was an important factor in the adhesion formation. As a result surgery with the gloves having powder should be avoided. The surgeon should be very careful in removal of contents from the peritoneum in colon operations and appendix perforations. It is recorded that the free peritoneal barriers such as Gelfoam, Surgicel increase the adhesion formation when used on the traumatized peritoneal surfaces (17).

Saglam et al (18) recorded that the abrasion alone did not lead to adhesion formation in the parietal peritoneum. The same result was confirmed in our

study and it was concluded that the loss of a peritoneal layer in one side did not lead to the adhesion formation.

It is also thought that the foreign body alone is not sufficient to produce adhesions and that the adhesion increases when it is together with a peritoneal ischemic damage such as abrasion (19,20). In this study we found the highest adhesion scores in the groups in which we used HGP, SRF and FRF with abrasion.

The infection was known to be an important factor in the adhesion formation (9). We also found higher incidence of adhesion formation in the FRF group with abrasion as a result of infection and foreign body granulomas were seen in both of the SRF and FRF groups. In these two groups the adhesion formation increased when abrasion was added ( $p<0.05$ ) (Table 2, 3). As a result peritoneal surface loss has an effect on the adhesion formation.

The effect of foreign bodies on the adhesion formation is explained by the antifibrinolytic mechanism. It is suggested that fibrinous adhesions could be dissolved and adhesion formation could not occur if the fibrinolytic mechanisms are not inhibited by the tissue ischemia and the foreign body (21). In our study we found the effects of HGP and sterile rat feces as foreign bodies on the adhesion formation similar ( $p>0.05$ ). We noted no significant difference between the FRF and SRF groups with and without abrasion. Although it is impossible to say that both of these cause the same amount of adhesion because of their unequal foreign body contents, we think that tie foreign body effect of feces is also important in the adhesion formation.

In conclusion we found that sterile rat feces caused adhesion formation by its foreign body effect, but the amount of adhesion increased in relation to the amount of foreign body and that the rate and severity of adhesion formation increased when peritoneal surface loss was also present.

#### Adezyon etyolojisi üzerine deneysel bir çalışma

*Adezyon oluşumunda yabancı cisimlerin, peritoneal yüzey kaybı ve infeksiyon gibi faktörlerin rolü olduğu bilinmektedir. Bu amaçla yabancı cisim etkisi yönünden steril edilmiş rat fecesini diğer bir yabancı cisim eldiven pudrası ile karşılaştırdık. Ayrıca adezyon oluşumunda infeksiyon faktörünü taze rat feçesi kullanarak inceledik. Deneyde 200-250 g ağırlığında toplam 90 rat 12 gruba ayrılarak çalışıldı. Bir gruba sadece laparotomi yapıldı. Eldiven pudrası süspansiyonu (EP) grubu; düşük, yüksek ve çok yüksek konsantrasyonda (YEP) olmak üzere üç grupta çalışıldı. Steril ve taze rat feçesi gruplarında da abrazyonsuz (SRFS, TRFS)*

ve abrazyonlu (Ab+SRFS, Ab+TRFS) olarak ikişer grupta çalışıldı. Abrazyon yapılanlarda her grupta 5'er ratta 10'ar model, abrazyon yapılmayanlarda ise her grupta 10'ar ratta 10'ar model oluşturuldu. Peritoneal abrazyon karın ön duvarında iki taraflı oluşturuldu. Postoperatif 14.gün relaparotomi yapılarak abrazyonlu gruplarda abrazyon yerine, abrazyonsuz gruplarda karaciğer alt yüz alanına olan adezyonlar derecelendirildi. Çalışmamızda sadece laparotomi, sadece abrazyon, abrazyonlu ve abrazyonsuz düşük konsantrasyonlu eldiven pudrası ve abrazyonsuz yüksek konsantrasyonlu eldiven pudrası süspansiyonu gruplarında adezyon gelişmedi. Çok yüksek konsantrasyonlu eldiven pudrası süspansiyonu gruplarında adezyon gelişmedi. Çok yüksek konsantrasyonlu eldiven pudrası süspansiyonuu grubu ile steril rat feçesi grubu yabancı cisim etkileri yönünden karşılaştırıldı. Ayrıca yine steril rat feçesinin yabancı cisim etkisi ile taze rat feçesinin kantitatif olmasa da infeksiyon etkisi ile birlikte yabancı cisim etkisinin adezyon üzerine etkileri karşılaştırıldı. YEP grubundan abrazyonsuz olanlarda  $0.8 \pm 0.8$ , abrazyonlu olanlarda (Ab+YEP)  $1.5 \pm 0.6$ , TRFS grubunda  $0.4 \pm 0.7$ , SRFS grubunda  $0.6 \pm 0.8$ , Ab+TRFS grubunda  $2.2 \pm 1.0$  ve Ab+SRFS grubunda  $2.2 \pm 1.0$  skorlarında adezyon görüldü. Adezyon skorları yönünden abrazyonsuz gruplardan SRFS, TRFS ve YEP ve abrazyonlu gruplardan Ab+SRFS ile Ab+TRFS grupları kendi aralarında karşılaştırıldığında fark bulunamadı ( $p > 0.05$ ). Adezyon skoru, Ab+TRFS ve Ab+SRFS gruplarında Ab+YEP gruplarına göre önemli derecede yüksek bulundu ( $p < 0.05$ ).

Çalışmamızın sonucunda rat feçesinin yabancı cisim olarak adezyon oluşturduğunu, peritoneal yüzey kaybı da olaya eklendiğinde adezyon oluşum oranının arttığını gördük. [Türk J Med Res 1994; 12(3): 97-102]

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