International Journal of Surgery 12 (2014) 1434–1438

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.journal-surgery.net

Original research

The effect of relaparotomy timing on wound healing in an animal model

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HIGHLIGHTS

- We investigated the effects of timing of the surgery through the previous incision.
- The relaparotomies were performed on the 3rd, 15th or the 30th postoperative days.
- The breaking strength of the wound scar decreases and musculoaponeurotic gap increases by time between the two surgeries.
- Collagen type I and III increase with relaparotomy compared to first laparotomy.
- Histologically increased fibrosis and tissue defects were detected by relaparotomy.

ARTICLE INFO

Article history:
Received 1 June 2014
Received in revised form
13 August 2014
Accepted 15 October 2014
Available online 30 October 2014

Keywords:
Relaparotomy
Wound healing
Incisional hernia
Tensile strength
Musculoaponeurotic gap

ABSTRACT

Background: The effect of the timing of the second laparotomy on wound healing is not clear. In an experimental study in rats, we aimed to investigate the effect of timing on wound healing after reoperations on the same surgical site. **Material and methods**: Forty-eight rats were divided into four groups. The control group (GC) didn't have another laparotomy whereas the relaparotomies on the same surgical site were performed either on the 3rd, 15th or the 30th postoperative days in the three study groups (G3, G15, G30 respectively). The midline tension pressure, collagen types I, III and, histological analysis were performed from the specimens in order to assess the wound healing and strength. **Results**: The tensile strength was the highest in GC and decreased gradually in G3, G15 and G30, the difference between the groups did not reach statistical significance. Higher collagen levels, increased fibrosis, and large defects were observed in relaparotomy groups than CG. The musculoaponeurotic gap was shortest in GC when compared to other three relaparotomy groups ($P < 0.001$) and, it was the longest in G30 ($P = 0.004$ between G3 and G30). **Conclusions**: Although non-statistically significant the gradual decrease in the tensile strength and the statistically significant increase in the musculoaponeurotic gap with time point out the importance of the timing of relaparotomy in the healing process. Early relaparotomies do not disrupt the healing process as much as relaparotomy performed later.

1. Introduction

Reinterventional laparotomies were performed after 0.5–15% of all laparotomies that the incidence is greatly affected by the type of surgery whereas the highest incidence is seen in gastrointestinal

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http://dx.doi.org/10.1016/j.ijsu.2014.10.013
1743-9191/

surgery [1]. Infection, disruption of anastomoses, wound dehiscence, hemorrhage, ischemia-necrosis, compartment syndrome, and intestinal obstruction are the most common causes of early urgent relaparatomies whereas definitive surgery after damage control or detection of persistent infection or infectious complications in cases of ongoing peritonitis can be the causes for planned relaparotomies [2].

Reusing the previous scar for the reoperation may change the healing process, unfortunately little is known about the effects of relaparotomies on the wound healing in abdominal incisions. Especially the effect of timing of the relaparotomy on wound healing and the risk of subsequent incisional hernia are not clear. The interval between the first laparotomy and re-laparotomy varies between cases, and the length of this interval may present different outcomes regarding wound healing and incisional hernia formation. In this study, we aimed to investigate the effects of timing of the reoperations through the previous midline incision on the abdominal wall healing. The primary outcome measure was abdominal wall healing as assessed by the measurements of breaking strength of the abdominal wound scar, the musculoaponeurotic gap and collagen types I and III levels. The secondary outcome measures were the development of incisional hernias, surgical site infection, abscess, hematoma, dehiscence or seroma formation in the examination of the surgical wounds.

2. Material and methods

The experimental protocol was approved by the Animal Ethics Review Committee of Gülhane Military Medical Academy, Ankara, Turkey. The study was sponsored by Scientific Research Committee of Diskapi Teaching and Research Hospital, Ankara, Turkey.

2.1. Animals

Forty-eight Sprague Dawley male rats weighing between 240 and 320 g were used. Male rats were used for standardization. They were housed at room temperature (20–24 °C), receiving photoperiod for 12 h and had free access to water and standard rodent chow. They were housed in Department of Laboratory Animal Health Center for Research & Development, GATA Medical School, Gülhane Military Medical Academy.

2.2. Study design

A median abdominal laparotomy and closure with an interrupted suture technique was performed in 48 rats. Animals were randomized by a computer generated list into experimental groups after completion of the first laparotomy. The sample was divided at random into 4 groups with 12 animals in each: group 1 (GC) as the control group; groups 2–4 (G3, G15, and G30) as the experiment groups. The control group (GC) didn't have another laparotomy whereas the relaparotomies were performed either on the 3rd, 15th or the 30th postoperative days in the three study groups (G3, G15, G30 respectively). The reoperations were performed in the same way through the previous incision. All the animals were sacrificed by an investigator blinded to the experimental groups 90 days after the last laparotomy of each group, the abdominal wounds were examined clinically for the presence of infection, seroma or incisional hernia and then specimens were obtained from the scar tissue of the midline incisions. The midline tension pressure, collagen types I, III and histological examination were performed by an investigator blinded to the experimental groups from the specimens in order to assess the wound healing and strength.

2.3. Surgery

Intraperitoneal anesthesia was performed with 60 mg/kg of ketamine–HCl (Ketalar, Pfizer, Eczacibasi, Istanbul, Turkey) and 10 mg/kg xylazine–HCl (Alfazyne, Alfasan, Woerden, Holland). Administration of 0.1 ml/100 g of the mixture of 7 ml ketamine (50 mg/ml) and 3 ml of xylazine (20 mg/ml) were performed. If required, repeated doses of 1/3 to 1/2 of the first dose were given. After shaving and disinfection of the abdomen with polyvinylpyrrolidone – active iodine 1%, a midline laparotomy on the linea alba was performed. The incision was 5 cm of length whose upper point was 1 cm below the xiphoid process. After inspection of peritoneal cavity, the surgical wound was closed in two layers. The peritoneum–muscleaponeurotic layer was closed through the interrupted technique by each stitch piercing at 0.5 cm of tissue on each side of the incision (tissue bites) and a stitch interval of 0.5 cm with 4/0 braided absorbable, polyglycolide-co-lactide suture (Pegelak, Dogsan, Trabzon, Turkey). The skin was closed in continuous style at tissue bites of 0.5 cm with 4/0 monofilament non-absorbable, polypropylene suture (Propilen, Dogsan, Trabzon, Turkey). The reoperations of G3, G15, and G30 were made with the same surgical technique on the same surgical site. The stitches and sutures of first surgery were removed and the presence or absence of incisional hernias was recorded before closure.

Three months after the last operation, euthanasia was performed by a lethal dose of ketamine and xylazine. The sutures of previous surgery from scar tissue were removed and clinical observation for hernia and infection was performed. Specimens of midline scar tissue (4 cm × 4 cm) with equal distance from the midline were resected half cm away from both cranial and caudal scar borders.

2.4. Traction resistance test

The fresh stripes of the abdominal wall pieces (4 cm × 1 cm) were prepared and both ends were fixed with metal clamps with the line of suture equidistant from the fixation points in order to adapt to traction resistance test in the tensile tester machine Tinius Olsen H5KS Model (Tinius Olsen Ltd., Surrey, England). The tensiometer consists of a board with two clamps, one fixed and the other free to slide following the movement of a strong electric motor, distracting the clamps. The rupture test rate was 30 cm/min and the load (kilogram force (kgf)) was recorded digitally on a personal computer until the point of tissue rupture.

2.5. Collagen analysis

Rat collagen type I Elisa kit (Catalog no. CSB-E08084r; Cusabio Biotech Co., Ltd., Wuhan, Hubei Province, China) and rat collagen type III Elisa kit (Catalog no. CSB-E07924r; Cusabio Biotech Co., Ltd., Wuhan, Hubei Province, China) were used for analysis of collagen types I and III respectively.

2.6. Histological analysis

Histological cuts were performed on the surgical piece fragments and were stained with picrosirius. The cuts were analyzed in optical microscope that emits polarized light, and the software Image Pro Plus was used to analyze the blades at the Experimental Pathology Laboratory – PUCPR. Collagen quantification and qualification were studied. The thicker and strongly birefringent collagenous fibers present a red-orange color (collagen I, mature) and the thinner and sparse fibers, slightly birefringent, present a greenish color (collagen III, immature). The percentage of 4 fields filled by the green and red fibers was measured. The addition of

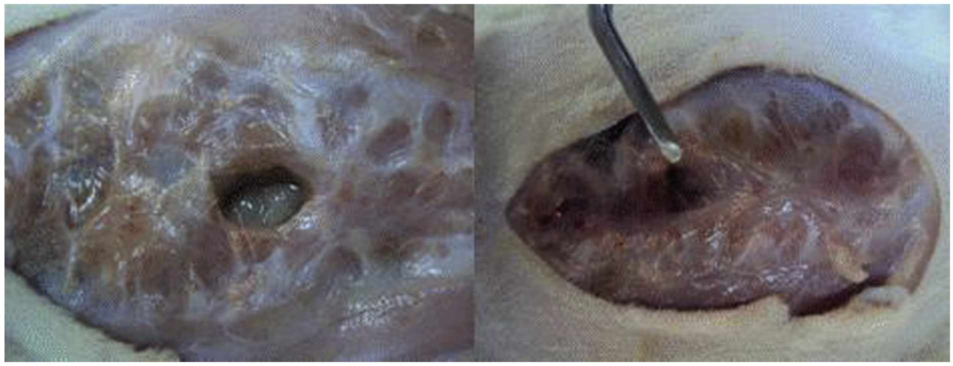

Fig. 1. Two incisional hernias; in G15 and in G30.

these two percentages was considered as the total collagen percentage of each one of the fields. An average of these percentages was obtained on each of the examined blades.

2.7. Statistical analysis

The results were analyzed statistically using SPSS 17.0 for Windows program (SPSS, Chicago, Illinois). Comparisons of dichotomous variables (presence of hernias) were made using the chi-square test with Yates' correction. The data symmetry condition was evaluated by the Kolmogorov–Smirnov test. The normally distributed data was presented as mean ± standard deviation. The results were submitted to statistical analysis by using the analysis of variance to compare the four groups. The multiple two-group comparisons were made by using the Bonferroni test. *P* values less than 0.05 were considered as significant.

3. Results

Two rats died in G15 twelve days after the second laparotomy. The inspectional examination of the surgical wounds of 46 animals revealed two incisional hernias, one in G15 (10%) and one in G30 (8%) ($P > 0.05$) (Fig. 1), whereas surgical site infection, abscess, hematoma, dehiscence or seroma formation were not detected in the examination of the surgical wounds. The tensiometric test showed that laparotomy wound scars in the control group had higher breaking strength values than relaparotomy groups (G3, G15, G30) and the tensile strength progressively decreased from G3 to G30 but the differences between the four groups in the tensile strength of stripes from the abdominal wall scars did not reach statistical significance (Table 1).

Higher collagen type I and type III levels were measured in relaparotomy (G3, G15, and G30) groups than GC but relaparotomy groups were not different from each other statistically. The musculoaponeurotic area gap was shortest in GC when compared to other three relaparotomy groups ($P < 0.001$) and, it was longest in G30 ($P = 0.004$ between G3 and G30) (Table 1). Histological examination revealed increased fibrosis and large defects in muscle tissue in relaparotomy groups (Fig. 2).

4. Discussion

These data suggests a declining trend by time in the breaking strength of the abdominal wound scar and shows statistically significant increase in the musculoaponeurotic gap as time elapses from the primary surgery to the relaparotomy. Collagen types I and III increase with relaparotomy compared to first laparotomy. Many issues regarding the method of the study and the assessment of the wound healing process will be discussed in order to be able to further comment on our results.

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers. This process may be interrupted at an early stage because of a planned laparotomy or an unplanned surgery because of various complications in the early postoperative period. This pause may take place in different postoperative days depending on the reason for relaparotomy. We have chosen different times fits the different phases of wound healing process to compare the effects of relaparotomy timing: the postoperative 3rd day when inflammation in the scar tissue is active and cellular proliferation is about to begin, the 15th day when contraction of the scar is at its maximum, and the 30th day when collagen synthesis start to decrease [3,4]. Although the subject of wound healing is not new, this study specifically tries to test the hypothesis stated: the effect of a second new incision on a previous wound that had already started the healing process.

Tensile strength is a commonly used method for measuring the progress of acute wound healing. Although the breaking strength steadily decreased as time elapsed from the primary surgery to the relaparotomy, the differences in tensile strength of the abdominal wall scars between the four groups with different relaparotomy

Table 1
The tensile strengths, musculoaponeurotic gap and collagen type I and type III levels of the stripes from the abdominal wall scars in the four groups. (Data is given as mean ± standard deviation).

Group	GC ($n = 12$)	G3 ($n = 12$)	G15 ($n = 10$)	G30 ($n = 12$)	*P*
Tensile strength (N/cm)	8.69 ± 7.01	7.17 ± 5.02	5.23 ± 2.19	4.74 ± 3.41	0.194
Gap	0.27 ± 0.05	1.92 ± 0.16[a]	2.22 ± 0.47[a]	2.36 ± 0.34[a,c]	<0.001
Collagen type I (pg/g)	233 ± 76	503 ± 173[b]	455 ± 190[d]	459 ± 196[d]	0.001
Collagen type III (pg/g)	363 ± 196	688 ± 224[d]	657 ± 223	713 ± 354[d]	0.007
Incisional hernia	0	0	1 (10%)	1 (8%)	>0.05

[a] $P < 0.001$ when compared to GC.
[b] $P = 0.001$ when compared to GC.
[c] $P = 0.004$ when compared to G3.
[d] $0.008 < P < 0.05$ when compared to GC.

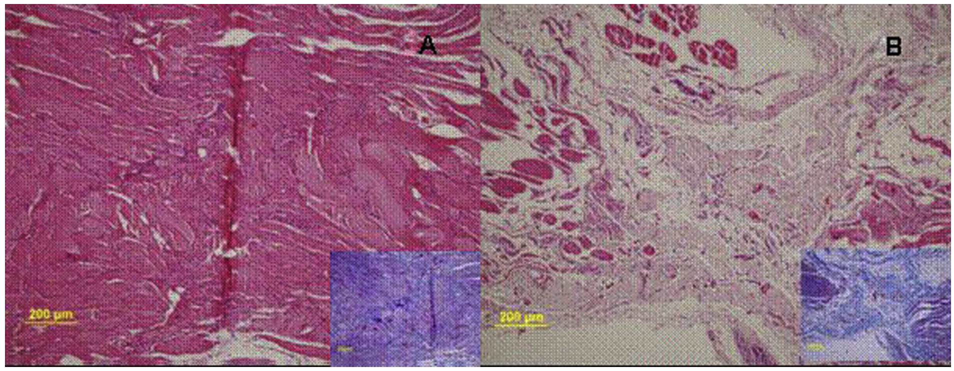

Fig. 2. A–B: (A,B; HEx100, little one-Trichromex100) Minimal damage level in control group (A), significant damage level and wide fibrotic defect in relaparotomy group (B).

times did not reach statistical significance. However the tensile mean strength value in subjects underwent relaparotomy at 30th day was only almost a half of the value in the control group with no relaparotomy. Tensile strength seems to decrease gradually by time.

Souza et al. have previously carried out a similar experimental study. They divided the rats into three groups: first group of rats had surgery on the first day, second group rats had surgery twice on the first day, and on the 30th day, and the third group had surgery three times on the first day, on the 30th day and on the 60th day [5]. Similar to our results, the authors noticed the absence of significant difference of breaking strength during the performance of the breaking strength study among the assessed groups. They found higher concentration of mature collagen (collagen type I) in the relaparotomy groups, compared to the control group, 30 days after the last operation. Therefore they stated that the resutures gained maturity faster than the primary sutures due to the fact that inflammatory reaction had already been started. They observed that in the three studied groups the concentration of total collagen and of the collagen fractions I and III was significantly different. However, the change in different fractions of collagen did not influence in the resistance gain of the scars, which developed in a similar way in the three groups, they studied [5].

In present study, collagen type I and type III levels were also measured 90 days after the last operation. This data showed increased collagen types I and III synthesis, especially in relaparotomy groups. Wound healing involves the coordinated expression of types I and III collagens. Initially, a provisional repair is characterized by predominantly type III collagen synthesis [6]. It has been recently reported that the control of collagen I transcription by the wound-associated macrophages is an early event and starts within the 2 days following injury and is not restricted to the remodeling phase of healing [7]. In incisional hernia patients, an increase of type III collagen proportionally to type I collagen has been determined and the impaired collagen metabolism is hypothesized to disturb the wound-healing process with the consequence of hernia disease and recurrence [6]. Collagen formation was investigated in seventy-eight prostheses implanted for inguinal and incisional hernia repairs for a mean period of 17.9 ± 11.2 (range 0.5–48) months [8]. The prostheses were explanted because of recurrence, chronic pain or infection. The authors concluded that the composition of scar tissue with a lowered collagen type I/III ratio and, therefore, reduced tensile strength might be a major contribution to hernia recurrence [8]. We did not find any difference regarding collagen type I/III ratio between the four groups. Impaired ratio of type I and type III collagen could probably be shown much later than our 90 day follow-up period as it has been measured prostheses explanted from hernia patients when they are symptomatic many months after the primary operation. Although our data did not include any inflammatory parameter, our results might suggest that it might be better if the wound healing is interrupted when inflammation is predominant, presumably during the first three days rather than being interrupted during the phase when collagen synthesis is predominant, presumably after the second week.

In the present study no early incisional hernia occurred in control subjects and 3rd day relaparotomy groups, whereas one incisional hernia was recorded in G15 and G30 groups. Whether more incisional hernias develop after a longer time may be predicted by using some parameters previously studied. In a retrospective study, the distance between the two rectus abdominis muscles was measured on computed tomography (CT) scans of the abdomen during the first postoperative months and several parameters were calculated to predict incisional hernia development. Incisional hernia occurrence was thus suggested to be predicted by measuring the maximum distance between the rectus abdominis muscles (RAM)s a postoperative CT scan [9]. In this study, we evaluated a histologic parameter similar to maximum distance between the RAMs – musculoaponeurotic gap – as an early marker. This gap was increased in relaparotomy groups and the increase became more prominent as time passed between the first and the second surgery in our study. The differences between different groups reoperated on different time points reached statistical significance when the musculoaponeurotic gap from the group reoperated on the earliest day (postoperative day 3) was compared with the group reoperated on the latest day (postoperative 30 days). Histologic examination also showed not only gaps between muscles but large defects throughout the muscle tissues in the relaparotomy groups. It is important to point out that further prospective studies are needed in order to assess the validity of musculoaponeurotic gap or presence of defects in muscle tissue in animal models and maximum distance between the RAMs in patients as predictors of incisional hernia development.

Suboptimal or defective wound healing may result in abdominal bursts or incisional hernias, both of which are more common after relaparotomies [10]. Although incisional hernia after open abdominal surgery remains a major cause of post-operative morbidity, there is no consensus in current practice of abdominal wall closure in elective surgery [11]. Besides looking for an answer to the question "May time of relaparotomy have an effect on wound healing?" the present study may have a potential clinical application about the timing of planned relaparotomies although we accept the rarity of the opportunity to choose the timing of a second laparotomy. There has been a debate on planned versus on demand relaparotomies in patients with secondary peritonitis [12–16]. Our study did not aim to compare on-demand versus planned relaparotomies or advocate the use of planned relaparatomies but to provide data about the effect of timing of relaparotomy on wound healing.

Limitations of our study are relatively short follow-up period for incisional hernia development and low number of animals per group to study the difference in the incidence of hernia development between the groups. A tissue biopsy for collagen estimation during the second laparotomy and comparing the collagen levels of second laparotomy with that of the tissue harvest would have been more illuminating to show whether it is a function of time or the effects of second laparotomy, although taking samples before the second laparotomy would have further complicated the surgical method and thus would have changed the outcome measures. It would also be interesting to create a model including more clinically applicable situations such as planned relaparotomies for ongoing peritonitis or damage control surgery. We did not plan such scenarios in order to not to complicate the hypothesis tested (whether late relaparotomy impair wound healing or not).

5. Conclusion

In conclusion, the gradual decreasing trend in the tensile strength values and collagen type I/type III ratios, the significant increase in the musculoaponeurotic gap with time point out that the interval between first laparotomy and relaparotomy may be of significance in wound healing process and the risk of subsequent incisional hernia formation. Relaparotomies on a later time than inflammation phase of the process may be related to poorer outcomes in wound healing. In clinical situations when the time of intervention cannot be optimized, measures should be taken to optimize the closure as the healing will be worse as time from the first laparotomy passes.

Ethical approval

The experimental protocol was approved by the Animal Ethics Review Committee of Gülhane Military Medical Academy, Ankara, Turkey.

Funding

The study was sponsored by Scientific Research Committee of Diskapi Teaching and Research Hospital, Ankara, Turkey.

Author contribution

MA conducted the experiments, collected data, wrote paper and drafted the manuscript.

ZE elaborated the ethical aspects of model building and conducted the animal surgery.

MK organized the research and collected the data.

ST conducted the collagen analysis of animal tissues.

MO planned and helped the collagen analysis.

AG conducted histological analysis and measured musculoaponeurotic gap.

HK conceived the topic and revised the manuscript for intellectual content.

TI provided animals, took care of the animals and took role in the surgery of the animals.

AS conducted Traction Resistance Test and analyzed this test.

ME analyzed data or performed statistical analysis.

OK interpreted the data and revised the final manuscript.

Conflict of interest

The authors declare that there are no any conflicts of interest.

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