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